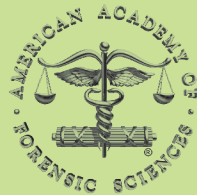


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*71<sup>st</sup> Annual Scientific Meeting  
Baltimore, MD  
February 18-23, 2019*



## AMERICAN ACADEMY OF FORENSIC SCIENCES

410 North 21<sup>st</sup> Street  
Colorado Springs, CO 80904  
Phone: (719) 636-1100  
Fax: (719) 636-1993  
Email: [info@aaafs.org](mailto:info@aaafs.org)  
Website: [www.aaafs.org](http://www.aaafs.org)

### ***PROCEEDINGS***

of the American Academy of Forensic Sciences 71<sup>st</sup> Annual Scientific Meeting

The *Proceedings* of the American Academy of Forensic Sciences is an official publication of the American Academy of Forensic Sciences (AAFS). It is devoted to the publication of the abstracts of technical oral papers and posters presented at the AAFS Annual Scientific Meeting. These include various branches of the forensic sciences such as anthropology, criminalistics, digital evidence, engineering, immunology, jurisprudence, odontology, pathology, psychiatry, questioned documents, and toxicology. Similar submissions dealing with forensic-oriented aspects of the social sciences are also included.

Please note that some of the abstracts included in the *Proceedings* deal with topics, results, and/or conclusions that are controversial. The publication of abstracts does not imply that the AAFS, its sections, or the individual section program chairs/committee members have verified or agree with the studies, results, and/or conclusions of each abstract. During the process of planning a scientific program, it is impossible to “peer-review” each abstract and presentation to the degree that is accomplished during manuscript review. Abstracts and presentations are accepted, in part, so they can be critiqued and reviewed by other scientists. Thus, a forum is created to discuss controversial issues.

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Articles from the AAFS *Proceedings* should be officially cited in the following format:

Fenton T.W., Kendell A.E., DeLand T.S., Haut R.C. Determination of Impact Direction Based on Fracture Patterns in Human Long Bones. *Proceedings of the American Academy of Forensic Sciences*, 64th Annual Scientific Meeting, Atlanta, GA. 2012. H72.

# ***PROCEEDINGS***

**of the American Academy of Forensic Sciences**

**February 2019**

**Volume XXV**

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### S1 Insane or Just Bad? The Anatomy of the Not Guilty by Reason of Insanity (NGRI) Defense

*Karen B. Rosenbaum, MD\**, New York, NY 10010; *Jan Seaman Kelly, BA*, Forensic Dynamics LLC, Las Vegas, NV 89147; *Christopher R. Thompson, MD\**, Los Angeles, CA 90024; *Daniel A. Martell, PhD\**, Park Dietz & Associates, Newport Beach, CA 92663; *Evan Van Leer Greenberg, JD\**, Van Leer & Greenberg Esq, New York, NY 10038; *Valerie Van Leer Greenberg, JD\**, Van Leer & Greenberg, New York, NY 10038; *Jennifer Piel, MD\**, Seattle, WA 98108; *Stephanie Domitrovich, JD, PhD\**, Sixth Judicial District of PA, Erie, PA 16501; *Susan Hatters-Friedman, MD\**, University of Auckland, Auckland, NEW ZEALAND

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**Learning Overview:** Attendees will have a greater understanding of what it means to establish a Not Guilty by Reason of Insanity (NGRI) defense, the risks and benefits of the defense, and a greater appreciation for issues facing the mentally ill in the criminal justice system.

**Impact on the Forensic Science Community:** The NGRI defense is not widely understood. This presentation will impact the forensic science community by helping to demystify the history and present state of the defense in the United States.

The theme for the 2019 American Academy of Forensic Sciences meeting is *Diligence, Dedication, and Devotion*. To illustrate this, the 2019 Interdisciplinary Symposium focuses on the diligence, dedication, and devotion that goes into presenting or opposing an NGRI defense. Recently, there has been more attention paid to the problem of mentally ill patients being treated in the criminal justice system. The symptoms and impairments most mentally ill defendants demonstrate do not make them an insanity defense. Even when the insanity defense is appropriately claimed, there is usually still controversy around its use.

The insanity defense is often misunderstood by the public because of misinformation and inaccurate media portrayals of the defense. Even within the court system, there is misunderstanding among judges, defense attorneys, and prosecutors as to what the defense means and when it is appropriate to use it, or even why the defense exists at all. This symposium will explain the history of the defense and will illustrate many aspects of the defense from the point of view of different court personnel and clinicians. Panelists will also discuss: criminal responsibility of juveniles, the role of neuropsychological testing, jury instructions, and the jury's perspective. Additionally, two experienced attorneys will provide their perspective on a recent high-profile case in New York City that took five-and-a-half years of intense diligence, dedication, and devotion to prepare for and try. A sitting judge (and member of the Jurisprudence Section) will speak on her experiences with this defense in Pennsylvania, and a forensic psychiatrist who has lived and practiced in New Zealand for many years will provide an international perspective via her experiences with the insanity defense and with high-profile cases.

A question-and-answer discussion session at the end of the symposium will enable attendees to ask questions of the speakers and for the speakers to ask questions of their fellow panelists. The goal of the panel is to educate attendees on questions of criminal responsibility as they pertain to people with significant mental health issues at the time of the crime and on juveniles, primarily by focusing on the NGRI defense.

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#### NGRI, Insanity, Psychiatry



### S2 Transitioning Young Scientists: Diligence, Dedication, and Devotion for Research and Casework Applications

*Alex J. Krotulski, MS\**, Center for Forensic Science Research & Education, Willow Grove, PA 19090; *Cheryl D. Hunter\**, Florissant, CO 80816; *Marcus Rogers, PhD\**, Purdue University, West Lafayette, IN 47907; *Nikolas P. Lemos, PhD\**, University of California - San Francisco, Palm Springs, CA 92262-6451; *Thomas W. Vastrick, BS\**, Apopka, FL 32703; *M. Eric Benbow, PhD\**, Michigan State University, East Lansing, MI 48824; *Sabra R. Botch-Jones, MS\**, Boston University School of Medicine, Boston, MA 02118; *Rudy J. Castellani, MD\**, Center for Neuropathology, Kalamazoo, MI 49008; *Jose R. Almirall, PhD\**, Florida International University, Miami, FL 33199; *Pamela M. Hofsass, MS\**, Contra Costa County Sheriff's Crime Laboratory, Martinez, CA 94553; *Rebecca A. Waxse, JD\**, Hugo, MN 55038; *Christine Funk, JD\**, Saint Paul, MN 55102; *Dean Michael De Crisce, MD\**, Special Treatment Unit, Avenel, NJ 07001; *Jennifer C. Love, PhD\**, Office of the Chief Medical Examiner, Washington, DC 20024; *Amanda R. Hale, MA\**, North Carolina State University, Raleigh, NC 27695; *Elisa N. Shoff, BS\**, Miami-Dade Medical Examiner Department, Miami, FL 33136

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**Learning Overview:** After attending this presentation, young scientists will better understand the disciplines contained within the American Academy of Forensic Sciences (AAFS) and the work being conducted within the respective fields. In addition, attendees will also learn about the importance of standards, innovative research, and casework in the continued development of each discipline.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating young and aspiring scientists on the steps needed to succeed as a forensic scientist and the importance of the work contained in each discipline. By providing attendees with a wide range of discussions from senior members of the forensic science community, young scientists will feel better prepared to succeed in the field of their choice and will be given the opportunity to interact with these speakers.

This year, the Young Forensic Scientists Forum (YFSF) has been restructured to better fit the needs and interests of both young scientists and the AAFS. This day-long session will focus on the foundation of forensic science and the commitment and qualities needed to pursue a successful career in most of the AAFS disciplines. Senior forensic scientists, spanning all disciplines, including current section chairs, will speak on topics including ethics, case studies, academia, novel research, etc. By compiling this unique group of presentations, we hope to appeal to young scientists from all disciplines, helping them integrate into the AAFS and beginning their journeys in forensic science.

At the end of the session, a résumé review will be conducted, giving young professionals an opportunity to seek one-on-one advice from prominent forensic scientists and representatives in their potential discipline. This time will also serve as a chance to network with peers and professionals and to develop valuable connections.

The YFSF will continue the tradition of hosting poster presentations as well as joining to the AAFS Bring Your Own Slides (BYOS) Session. The YFSF poster presentations will be held in conjunction with the AAFS Academy-wide poster sessions on Wednesday, Thursday, and Friday, with a special designation in the program for those presenting on behalf of the YFSF. The BYOS Session will be integrated into the AAFS BYOS session and will focus on interesting and unique case reports from young scientists in the field. If you are interested in presenting during the BYOS Session, please complete the abstract form in your welcome bag.

The YFSF looks forward to hosting all of these exciting and educational upcoming events. Our mission is to encourage education and career development through presentations and mentoring of young scientists, including young professionals and graduate or undergraduate students.

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#### YFSF, Education, Forensic Science



## Breakfast Seminars – 2019

### BS1 Checklists and the Scientific Method: Will Lawyers Make You Eat Your Checklists for Breakfast?

*Terry-Dawn Hewitt, LL.M.\*, McKenna Hewitt, Denver, CO 80224-3703; Wayne J. McKenna, LL.B.\*, McKenna Hewitt, Denver, CO 80224-3703*

**Learning Overview:** The purpose of this presentation is to stimulate thought and discussion among attendees regarding the pros and cons for experts who use checklists to help them investigate or analyze issues that may end up in court. Attendees will learn some of the benefits for those experts who develop and utilize checklists as an aid in applying the scientific method. Attendees will also be introduced to some of the risks to such experts from litigation strategies employed by opposing lawyers who obtain access to these checklists. The goal of this presentation is to demonstrate that the benefits of checklists can outweigh the litigation risks, which can be managed and may be inevitable in any event.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping those involved with civil or criminal cases develop strategies for dealing with checklists in litigation. This presentation will explain why checklist development by trial lawyers for the purposes of challenging forensic experts is to be expected, using examples from the field of fire investigation. With this perspective, members of each forensic science discipline are encouraged to take steps to begin the thoughtful development and implementation of checklists based on their industry standards. In this way, they can begin obtaining the benefits of such checklists while implementing measures to manage the litigation risks.

In 2017, the National Commission on Forensic Science approved a Views Document on the *Use of Checklists in Forensic Science*, indicating that it may be beneficial to pursue scientific studies into the employment of checklists in various aspects of the forensic sciences.<sup>1</sup> This Views Document cites examples of the successful use of checklists in the fields of health care and aviation, as well as in the criminal justice system. It also hypothesizes that when properly structured, checklists can help certain forensic science applications by reducing human error and bias.

While scientific studies into the pros and cons of using checklists, as suggested by the Commission, is a sound idea, the issue is whether forensic experts should hold off on utilizing checklists until the results of any such scientific studies are published. Alternatively, should experts consider immediately exploring the use of checklists in their fields? Why? Because lawyers are already developing and using checklists to challenge expert witnesses.

The proliferation of consensus standards in each forensic discipline empowers lawyers (with the help of their own experts) to develop checklists based on these standards. These checklists can simplify an attorney's task of finding shortcomings or bias in the work of forensic experts. Therefore, in this world of increasing standardization, the strategy of failing or refusing to develop or use checklists will not shield experts from having competent counsel use checklists to test the expert on his or her competence, thoroughness, or bias. By being checklist-adverse, experts cannot avoid the associated litigation risks. Conversely, they may fail to experience the benefits checklists have to offer.<sup>1,2</sup> It is reasonable for experts to be concerned about criticisms that can arise from items on checklists that go unaddressed. However, by planning ahead, strategies are available to counter such attacks.

Experts and their organizations will ultimately have to conduct their own risk-benefit analysis concerning the use of checklists. Assuming checklist development by trial attorneys is likely, members of each forensic science discipline can get ahead of this curve in three ways. First, by taking steps to determine the best application of checklists in their practice. Next, by getting plans underway to develop and revise checklists based on industry standards. Finally, by being mindful of litigation challenges that can be based on these checklists, with a view to including risk management training and tactics for the experts who use them.

#### Reference(s):

- <sup>1</sup> National Commission on Forensic Science, Views of the Commission: Use of Checklists in Forensic Science. (Adopted January 9, 2017). Washington, DC. Accessed July 30, 2017. <https://www.justice.gov/archives/ncfs/page/file/934416/download>.
- <sup>2</sup> Gawande, Atul. *The Checklist Manifesto: How to Get Things Right*. Reprint edition. New York, NY: Picador, 2011.

#### Checklists, Fire Investigation, Consensus Standards



### BS2 *Voir Dire*: Is It Science or Is It “Bull”—A Historical Perspective and Comparative Analysis

Patricia C. Smith, MSL\*, Harris County District Attorney's Office, Houston, TX 77002; Patricia Williams, MS, RN, Cambria, CA 93428; Randall Smith, BSME, Houston, TX 77014

**Learning Overview:** The goal of this presentation is to encourage dialogue about *voir dire* and explore what the scientific and legal communities can do to ensure an unbiased jury.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reaching out to other disciplines to promote jury service and encourage a fair and unbiased jury selection process through enhanced research, testing, and analysis.

The United States Constitution ensures the Defendant's right to a jury trial.<sup>1</sup> The United States is not the only country that engages in jury trials. Great Britain, Canada, Australia, New Zealand, and more than 40 other nations employ juries of citizens drawn from the general population who decide cases collectively.<sup>2</sup> Scotland, Wales, and Canada do not give the right to a jury trial to defendants “... for which the prescribed punishment is less than two years in prison.”<sup>3</sup> However, 80% of all jury trials worldwide take place in the United States.<sup>4</sup>

The makeup of a jury consists of judges, lay persons, citizens, and experts who are utilized in other countries depending on the type of case. Historically, jury selection excludes those new to the area, non-citizens, advanced age, poor health, non-voters, and non-drivers. Some people are automatically excluded (for example, Los Angeles County Probation Officers are excused from jury duty once they fill out the jury service survey).

A jury's role is to render a verdict based on evidence presented at trial. Each juror is to be convinced beyond a reasonable doubt. There is no clear definition of reasonable doubt and many legal scholars, judges, and lawyers will not attempt to define it. Some legal reactionaries have opined a preponderance of the evidence is loosely quantifiable as >=51% and beyond a reasonable doubt is more than that. How can a jury render a fair and unbiased verdict if the jury does not know or understand what reasonable doubt is? Unfortunately, it is this lack of clarity that opens the door for jury misconduct.

Our Founding Fathers were very familiar “with a long list of government abuses from English history ... [where] the English monarchy had punished, tortured, and killed people for everything from disagreeing with the king to choosing a different religious path from the government's approved denomination;” a practice that resulted in an increase in jury misconduct.<sup>5</sup> Therefore, is society picking “the lesser of two evils” and should reaching a jury verdict supersede the attempt of perfect justice?

The Lord Mansfield's Rule from 1785 said “jurors could not impeach their verdict by making affidavits or otherwise testifying as to their own misconduct.”<sup>6</sup> However, in 1905, the Texas Legislature allowed jurors to testify during hearings based on jury misconduct.<sup>6</sup> The idea of cases overturned because of a juror's misconduct or allegations of a juror's misconduct has fueled the notion for the need of criminal justice reform. More specifically, the need for scientific jury selection or professional jurors may be the solution.

People have become desensitized after decades of watching television shows and movies about police officers, lawyers, and doctors. Is it possible professional jurors already exist? Are jurors more likely created by clever lawyers and jury consultants? The following is a list of cases, television shows, and movies involving jury misconduct that may have been prevented through carefully constructed *voir dire* or utilizing trial consultants differently:

- *People of the State of California v. Orenthal James Simpson*
- *Lloyd v. Johnson & Johnson*
- *State of Florida v. John Goodman*
- Television show, *Bull*
- Motion picture, *Runaway Jury*<sup>7</sup>
- *12 Angry Men* (1957) and *Twelve Angry Men* (1997)<sup>8,9</sup>

Ultimately, there has been little to no research determining that utilizing consultants directly or indirectly adversely affected *voir dire* or jury verdicts.

#### Reference(s):

1. 6<sup>th</sup> Amendment, United States Constitution.
2. Hans, Valerie P. “Jury Systems Around the World” (2008). *Cornell Law Faculty Publications*. Paper 305. <http://scholarship.law.cornell.edu/facpub.305>.
3. Hans, Valerie P. & Neil Vidmar. *Judging the Jury*. Perseus Publishing: Cambridge, Massachusetts 1986.
4. Hans, Valerie P. & Neil Vidmar. *Judging the Jury*. Perseus Publishing: Cambridge, Massachusetts 1986.
5. No author. *Revolutionary War and Beyond*. Only, no date. Retrieved July 24, 2018. [www.revolutionary-war-and-beyond.com](http://www.revolutionary-war-and-beyond.com).
6. Keltner, David. *Jury Misconduct in Texas: Trying the Trier of Fact*, 34 SW L.J.1131 (1980). Retrieved July 24, 2018. <https://scholar.smu.edu/smulr/vol34/iss5/3>.
7. *Runaway Jury*, directed by Gary Fleder (2003, New Orleans, LA: Regency Enterprises, 2009). DVD.
8. *12 Angry Men* (1957), directed by Sidney Lumet (1957, Los Angeles, CA: United Artist, 2011). DVD.
9. *Twelve Angry Men* (1997), directed by Williams Friedkin (1997, Los Angeles, CA: MGM, 1997). DVD.

#### *Voir Dire*, Jury, Trial Consultants

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## Breakfast Seminars – 2019

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### BS3 Digging Up New Standards for Forensics: An Update on Standards Developing Organizations (SDOs)

Karen Reczek, MLS\*, NIST, Gaithersburg, MD 20899; Teresa Ambrosius, BA\*, AAFS, Colorado Springs, CO 80904

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**Learning Overview:** After attending this presentation, attendees will: (1) learn about advances in forensic science standardization from United States SDOs, including the American Academy of Forensic Sciences (AAFS) Standards Board (ASB), the American Society for Testing and Materials (ASTM) International, the American Dental Association (ADA), and the National Fire Protection Association (NFPA), as well as international SDOs, and the International Organization for Standardization (ISO); and (2) gain a better understanding of the progress of documentary standards development in different forensic science disciplines, including fire debris, seized drugs, biology, digital evidence, toxicology, friction ridge, blood stain pattern, footwear/tire tread, crime scene investigation, and fire investigation.

**Impact on the Forensic Science Community:** The 2009 Report by the National Research Council stressed the lack of standardization in forensics.<sup>1</sup> The advent of the Organization of Scientific Area Committees (OSAC) for Forensic Science, administered by the National Institute of Standards and Technology (NIST), has resulted in several years of concerted efforts to identify gaps in standards and begin their development using the voluntary consensus standards development process. This presentation will impact the forensic science community by making attendees aware of new and emerging standards in the various forensic science disciplines and how they can be implemented in the laboratory setting, incorporated into quality manuals, be applied to accreditation scopes, and can assist in further validation of methods and techniques in interlaboratory studies.

Presenters will provide an overview of recent progress in standards development in forensic sciences. In 2009, the National Research Council Report, *Strengthening Forensic Science in the United States: A Path Forward*, summarized the challenges facing the forensic science community and the lack of mandatory standardization, certification, and accreditation.<sup>1</sup> From this Report, the Department of Justice (DOJ) and the NIST signed a Memorandum of Understanding which led to the establishment of the National Commission on Forensic Science (NCFS) and the NIST-administered OSAC for Forensic Science. OSAC has been working closely with various SDOs to bring the community together to define requirements for the various disciplines for submission to the standards development process.

Learn about standards currently in development, soon to be published, and new standards from various SDOs, such as ASB, ASTM International, ISO, the American Dental Association (ADA), and the NFPA. New standards include interdisciplinary topics, such as evidence collection and labeling, interpretation, and reporting, and discipline-specific standards in the areas of criminalistics, digital evidence, facial recognition, photography, biology, pattern evidence, and crime and fire scene investigation, with a focus on terminology, measurement uncertainty, measurement traceability, quality assurance, and calibration.

#### Reference(s):

- <sup>1</sup> National Academy of Sciences, National Research Council, *Strengthening Forensic Science in the United States: A Path Forward*, Washington, DC: National Academies Press, 2009.

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#### Standards, Development, Standards Developing Organizations





## Breakfast Seminars – 2019

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### BS4 The American Academy of Forensic Sciences (AAFS) Responds to the Opioid Crisis

*Barry K. Logan, PhD\*, NMS Labs/CFSRE, Willow Grove, PA 19090; Agnes D. Winokur, MS\*, DEA/Southeast Laboratory, Miami, FL 33182; Victor W. Weedn, MD, JD\*, George Washington University, Washington, DC 20007; Michael F. Rieders, PhD\*, NMS Labs, Willow Grove, PA 19090; Bruce A. Goldberger, PhD\*, University of Florida College of Medicine, Gainesville, FL 32608*

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**Learning Overview:** After attending this presentation, attendees will be able to describe how the AAFS responded to the impact of the ongoing opioid crisis on the forensic sciences by creating a presidential committee to align the efforts of the AAFS sections impacted by the workload, resulting in the increase in forensic examinations related to the investigation of opioid-related crimes and fatalities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a key role for the AAFS in coordinating the community's response to external events that impact the profession. Specifically, this presentation will demonstrate that AAFS members and the organization have played a role in developing and implementing strategies designed to counter the effects of the opioid crisis on the practice of forensic science.

The AAFS Ad Hoc Committee on Synthetic Opioids was established in 2017 in an effort to evaluate the multidisciplinary effects of the opioid crisis on the forensic sciences. The members of the committee represent the most impacted disciplines and their unique perspectives are used to increase awareness and better our understanding of the opioid crisis in the fields of criminalistics, forensic pathology, toxicology, and pharmacology.

In the past year, synthetic opioid-related non-fatal and fatal overdoses have continued to increase, specifically those associated with fentanyl analogs. This fentanyl threat is multi-faceted and requires the collaborative efforts of various forensic disciplines, including chemistry, pathology, and toxicology. In a collective effort, the AAFS Ad Hoc Committee on Synthetic Opioids has created four subcommittees to gather diverse resources and focus them in a coordinated and targeted approach. Experts will provide an update on the four subcommittees as well as share their experiences and perspectives. The subcommittees are: (1) the Advocacy Subcommittee which tracks congressional legislation and budget proposals that would influence the forensic sciences related to the opioid crisis; (2) the Information-Sharing Subcommittee which acts as a clearinghouse for the activities of various allied professional organizations, including the Association of Public Health Laboratories (APHL), the American Industrial Hygiene Association (AIHA), the Centers for Disease Control and Prevention (CDC), the Consortium of Forensic Science Organizations (CFSO), the American Association of Crime Lab Directors (ASCLD), and others; (3) the AAFS-Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Resource Landing Page Subcommittee which works to identify and list valuable resources that can be linked to the AAFS-SWGDRUG Landing Page and used by the forensic community in their efforts to combat the opioid epidemic in the United States; and (4) the American Society for Testing and Materials (ASTM) Standard Practice Subcommittee which has identified a need for standards that would help forensic practitioners (federal, state, local, and private) overcome the analytical challenges associated with the detection, identification, and reporting of novel and emerging opioids. The group seeks to work with ASTM and other organizations in creating standards that provide guidelines for the analysis of opioids, as well as a standard for naming these novel and emerging substances.

This breakfast session will highlight the activities of the AAFS Ad Hoc Committee on Synthetic Opioids with a focus on sharing identified resources, solutions, and best practices with the AAFS membership.

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### Synthetic Opioids, Fentanyl Analogs, Legislation



### BS5 Dining With DNA: Extracting Usable Profiles From Burned Bone

*Katelyn L. Bolhofner, PhD, Texas Tech University, Lubbock, TX 79410; Matthew V. Emery, PhD, Arizona State University, Tempe, AZ 85281; Jane E. Buikstra, PhD, Arizona State University, Tempe, AZ 85287-2402; Laura C. Fulginiti, PhD, Forensic Science Center, Phoenix, AZ 85007; Anne Stone, PhD\*, ASU School of Human Evolution & Social Change, Tempe, AZ 85287-2402*

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**Learning Overview:** The major goal of this presentation is to offer best-practice procedures for the recovery of samples from burned skeletal remains for the purpose of recovering DNA for identification purposes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a predictive model for determining which samples from incinerated skeletal remains are likely to produce the best results for Short Tandem Repeat (STR) testing (expanded Combined DNA Index System (CODIS) loci with miniSTRs), for mitochondrial Genome (mtGenome) sequencing, and for genome-wide Single Nucleotide Polymorphism (SNP) analysis given the extent of charring and other visible signs reflecting the level of burning.

Individuals who die before or during a fire often are quite difficult to identify due to the obliteration of typical characteristics useful for scientific identification. While DNA analyses have become central to forensic science, with applications ranging from unidentified decedents to trace evidence, burned bone analysis remains elusive. Extracting DNA from these samples presents a special challenge to medicolegal investigations. Fire and heat cause major alterations in soft tissue and bone, with cremation or near cremation producing unusable samples for DNA profiling. Or so it was previously thought! New methods for ancient DNA extraction, DNA library construction, and targeted enrichment developed during the last two to five years have great promise for such forensic applications. In ancient contexts, these methods have been applied to: (1) conserve (and effectively immortalize) DNA from small and/or degraded samples, enabling multiple analyses without exhausting the sample and allowing additional analyses to be performed in the future; and (2) target a large genome region or number of SNPs (such as the complete mtGenome or genome-wide SNPs) for subsequent Next Generation Sequencing (NGS). This seminar will highlight the utility of applying this methodology to modern forensic samples with the express intent of increasing scientific identifications.

Over a period of two years, this study obtained and documented 80 samples from 27 fire death and cremation cases. Samples were chosen with the goal of obtaining statistically significant results from different skeletal tissues showing different levels of burning. From these, DNA was successfully extracted from 68 samples (including blanks) using two different DNA extraction protocols: one currently used to obtain DNA from ancient remains and another used in forensic DNA analysis. This presentation will report the success of this sampling strategy, noting the degree to which visible markers upon thermally or otherwise altered human remains (here, bones and teeth) can be used to predict DNA preservation, and thus inform project design for sample selection. This presentation will discuss this process, offering the first evidence for optimal extraction practices for burned skeletal remains recovered from fire-related forensic contexts.

Using modern forensic cases to illustrate, this seminar will offer evidence-based best practice procedures for the analysis of human remains with burned DNA, answering the call of the National Research Council of the National Academies 2009 Report, *Strengthening Forensic Science in the United States: A Path Forward*, to develop reliable and accurate scientific methods. Further, this presentation will encourage collaborations between academic institutions and medical examiner/coroner offices nationally and internationally.

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### Degraded DNA, Burned/Cremated Bone, DNA Profiling



### BS6 Undetermined: The Devil Is in the Details

James J.C.U. Downs, MD\*, *forensX, LLC, Savannah, GA 31406*; Andrew M. Baker, MD, *Hennepin County ME, Minneapolis, MN 55415*

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**Learning Overview:** The goal of this presentation is to familiarize attendees with the necessity of considering medical history and scene investigation in determining cause and manner of death and/or in evaluating injuries. After attending this presentation, attendees will understand that better investigation can provide additional answers, but that not all cases are solvable.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting ways to improve forensic pathology analysis of case materials while recognizing that sometimes not all answers are forthcoming.

The art and science of determining cause and manner of death is a challenge for the forensic pathologist—both the novice and veteran practitioner. The five established universal manners of death include “undetermined” to reflect that an answer is not always possible to a reasonable degree of certainty. As many as five percent of all medical examiner cases are eventually classified as “undetermined.” Some embrace the diagnosis, arguing that “you can’t be wrong with undetermined.” In such situations, resistance and frustration are common, since interested parties may feel that the physician “took the easy way out” by not having an opinion. This may be, in part, due to misunderstanding what message is being conveyed by the term. Confusion may be further compounded when the cause of death is also not readily apparent.

The designation “undetermined” applies not only as a cause and manner of death but can also apply when determining how events happened when such questions arise. In medically evaluating death and/or injury, context can be critical. The circumstances are provided by the history and the scene. Failure to find and/or fully appreciate the answer(s) tends to occur in situations in which there is insufficient investigation, scene evaluation, or medical history. Thus, for a medical examiner, scrutinizing the crime scene (not necessarily just a death scene) can be invaluable.

Frustrations may arise because two equally qualified physicians may interpret case materials differently and arrive at different answers. In reality, different experiences and different qualifications may lead to alternate theories, diagnoses, and opinions. Forensic pathology is *medicine* at its core—not a bench science. Medicine applies various scientific disciplines to a biological context—the individual patient. A *sine qua non* of competent medical practice is history, which provides essential context for case evaluation, culminating in a medical diagnosis. The essential nature of medical history is illustrated by the need for federal legislation (the *Health Insurance Portability and Accountability Act*) attempting to ensure medical records access and privacy.<sup>1</sup> Medically, the scene circumstances and injury are a big part of that history.

Through a review of several cases involving deceased and living patients, this presentation will point out where a different and/or a second point of view has proved valuable in clarifying how injuries occurred, allowing a more definitive conclusion regarding the nature of sudden unexpected death and/or injury. Practitioners should accept that there will always be cases that end up as undetermined despite lengthy investigation and innumerable analyses, but the “there’s no harm in calling it undetermined” philosophy should be rejected as the fallacy that it is.

#### Reference(s):

<sup>1</sup>. [https://en.wikipedia.org/wiki/Health\\_Insurance\\_Portability\\_and\\_Accountability\\_Act](https://en.wikipedia.org/wiki/Health_Insurance_Portability_and_Accountability_Act).

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#### Undetermined, Medical History, Scene Investigation



### BS7 The Exoneration of Kirstin Blaise Lobato

*Vanessa Potkin, JD\*, Innocence Project, New York, NY 10013; Jane Pucher, JD\*, Innocence Project, New York, NY 10013; Jeffery K. Tomberlin, PhD\*, Dept of Entomology, College Station, TX 77843-2475; Andrew M. Baker, MD\*, Hennepin County ME, Minneapolis, MN 55415*

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**Learning Overview:** The goals of this presentation are to help attendees: (1) understand the factors that lead to wrongful conviction; (2) describe the features used to estimate time of death; and (3) understand the role of forensic entomology in narrowing time-of-death estimations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the numerous factors that led to the wrongful conviction of a young woman and years of imprisonment for a murder she did not commit. Attendees will appreciate the advantages of a second opinion in cases in which time of death may be an issue and the importance of considering another forensic science that was not considered at the time of original trial.

In most death investigations, time of death is not a critical issue in court. However, for those accused of homicide—especially those who have an alibi for a specified time period—the ability to narrow the time frame in which death likely occurred can mean the difference between exculpation and wrongful imprisonment. Oftentimes medical examiners may be unaware that time of death is of particular significance in a case, unless or until a lawyer informs them.

On the night of July 8, 2001, around 10:00 p.m., a man’s body was discovered covered with trash beside a dumpster in a parking lot in Las Vegas, NV. The victim had been mutilated: his teeth were knocked out, he suffered numerous stab wounds, his skull was fractured, and his penis had been severed from his body. Police investigation ultimately turned to Kirstin Blaise Lobato (“Blaise”), a slight eighteen-year-old girl from a town several hours from Las Vegas. Blaise had no connection to the victim; rather, she was implicated solely based on rumors that she had cut a different man’s groin when he tried to attack her in a motel parking lot; however, that attack occurred several weeks *before* this July 8 homicide.

Blaise had an alibi for the majority of July 8—it was undisputed she was in her hometown, several hours from Las Vegas, after 11:00 a.m. and for the rest of that day. Under the State’s theory at trial, Blaise killed the victim during the early morning hours of July 8. This meant that the victim’s body laid undiscovered in the daytime summer heat for more than 12 hours, from the early morning (when Blaise allegedly killed him) until his body was found at 10:00 p.m. The State relied heavily on a medical examiner’s time-of-death estimation that the victim could have died up to 24 hours before he was pronounced dead at 3:50 a.m. on July 9, meaning at approximately 4:00 a.m. on July 8. The State argued that this put the victim’s death at a time when Blaise was not accounted for by her alibi witnesses, and the jury convicted her.

During Blaise’s post-conviction litigation, this time-of-death estimate was revisited. A consulting pathologist noted that the decedent was initially examined and found to be in full rigor mortis at 3:50 a.m. Rigor mortis had disappeared by the time the autopsy commenced at noon, eight hours later. Given the rapidity with which the decedent went out of full rigor, and given the outdoor temperatures in Las Vegas in July, the pathologist estimated that the decedent most likely died within several hours of 8:00 p.m. on July 8—long after Blaise had left Las Vegas. Three renowned forensic entomologists independently reviewed the crime scene and autopsy evidence and found an absence of blow fly activity. Blow flies are early colonizers of dead bodies, and the conditions in which the victim’s body was found were ideal for rapid colonization: outdoors, in a hot climate, surrounded by garbage. All three entomologists independently concluded that, based on the absence of blow fly colonization, the victim was likely killed after 8:00 p.m. on July 8, 2001, during which time Blaise was, as even the State conceded at trial, hours away from Las Vegas.

Based on these findings, as presented during an evidentiary hearing, the presiding judge vacated Blaise’s conviction. The judge found that her trial attorneys provided ineffective assistance of counsel in failing to consult with a forensic pathologist as well as with a forensic entomologist, whose consult would have narrowed the time-of-death estimate to a time frame during which Blaise could not possibly have committed the crime.

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### Exoneration, Time of Death, Blow Flies



### **BS8 The National Institute of Justice (NIJ) Cold Case Working Group and Its Best Practices Guide for Implementing and Sustaining a Cold Case Unit in Police Agencies: A Path Forward to Solving the Cold Case Crisis**

*James M. Adcock, PhD\*, Collierville, TN 38017; Thomas C. McAndrew, MA\*, Pennsylvania State Police, Orefield, PA 18069; Charles M. Heurich, MFS\*, National Institute of Justice, Washington, DC 20531*

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**Learning Overview:** After attending this presentation, attendees will better understand the status of cold case investigations in the United States and be more familiar with the recommendations found in the newly published NIJ Best Practices guide for implementing and sustaining a cold case unit in police agencies.<sup>1</sup> Attendees will also be aware of how these guidelines will affect ongoing homicide and cold case investigations and will be presented with a novel approach, utilizing experience and donated funds, along with the NIJ guide's recommendations, to help these police agencies implement and sustain the process, furthering the intent of the NIJ cold case guide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community primarily by outlining recommendations that will help police agencies better address the cold case crisis in America. Attendees will see research-supported concepts along with application guidelines on how to create, implement, and sustain a proper cold case unit. This process, coupled with the efforts of a community-based non-profit corporation, will produce maximum effectiveness through the solving of more cold cases while at the same time increasing the clearances of other homicides. The example given should be an incentive for other communities to follow.

From 1980 to 2016, the United States accumulated more than 242,000 cold cases or unresolved homicides.<sup>2</sup> Then, in 2016, the Federal Bureau of Investigation (FBI) Uniform Crime Reports (UCR) reported our national clearance rate of homicides at 59.4%, the lowest in our history, while many cities saw a significant rise in homicide incidents.<sup>3</sup> These unsolved homicides have, for the most part, just been added to the cold case status that is rising by the thousands each year.

In 2015, the NIJ started the Cold Case Working Group with the intent of formulating a "best practices" guide for the implementation and sustainment of a cold case unit in police departments. (At this writing, the NIJ document is scheduled to be published in the fall of 2018.) For many years prior to this working group, NIJ had funded millions of dollars for agencies to work cold cases through their (NIJ) DNA Cold Case Grants. While they were productive, it became clear that more research and work was needed to make the system better; therefore, the working group.

While there are approximately 50 recommendations in this manual, this presentation will only highlight certain ones that appear to have the most impact on police decision-making regarding their attempts to solve cold cases. The latest draft of the guide explicitly states that we are in a "cold case crisis"; that agencies must conduct a complete inventory of all unresolved cases along with associated evidence; that only a "dedicated" unit will provide maximum effectiveness with increased clearances; that the use of "vetted" outside sources are important to saving time and manpower; and, as a path forward, that sustaining the unit over time is critical to the solving of homicides and cold cases on a regular basis.<sup>3</sup>

Many police agencies suffer from manpower losses and insufficient funds to even consider implementing a sustainable cold case unit. These are all obstacles for the police supervisors to overcome. The first and foremost decision must be a commitment to have a "dedicated" cold case unit. Then, if the guide is to be a productive document, how does an agency get over the hump regarding manpower and funding and manage to sustain a viable unit? Hopefully, the funding from NIJ for cold cases will be reinstated, but experience has shown that many times after the funds are depleted (one to two years), the cold case unit is dismantled, which defeats the "sustainment" intent of the process.

Detectives spend approximately 60% of their time conducting administrative duties. That, coupled with manpower shortages, can be partially resolved through the "vetting" of outside sources, such as retired detectives, professors, other professionals from the community, and grad students. These sources should come from the community at large and, when vetted by the police agency, can accomplish most of the administrative tasks that detectives would normally perform, leaving them to do what they do best—investigate.

Lastly, a community-based non-profit such as the Mid-South Cold Case Initiative could be a partial solution to the sustainment concern by funding the cold case unit over time. This corporation was specifically designed to raise funds in the mid-south that are earmarked specifically for police agencies to fund a dedicated cold case unit.

In conclusion, the design of the best practices guide is geared to help police agencies to not only grasp the concept of investigating cold cases for justice, families, and the safety of our communities, but to also prevent some communities from having a crisis that will drain their resources to the limits. The process of investigating cold cases is an intricate part of the equation to solving homicides while attacking the issue from both ends of the spectrum simultaneously, hot and cold.

#### **Reference(s):**

- <sup>1</sup> National Institute of Justice (NIJ). *National Best Practices for Implementing and Sustaining a Cold Case Investigation Unit*. Latest draft April 2018.
- <sup>2</sup> Murder Accountability Project, [www.murderdata.org](http://www.murderdata.org); accessed last July 2018.
- <sup>3</sup> UCR 2016, [www.FBI.gov](http://www.FBI.gov); accessed July 2018.

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#### **Cold Cases, Unresolved Homicides, Best Practices**



## Luncheon Seminar – 2019

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### L1 Forensic Sciences Without Borders: Forensic Sciences Services in Brazil

*Heather E. McKiernan, MSFS\*, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Joao Carlos L. Ambrosio, MSc\*, Brazilian Federal Police, Brasilia, Distrito Federal 70610-200, BRAZIL; Taís R. Fiorentin, PhD, Willow Grove, PA 19090; Barry K. Logan, PhD\*, NMS Labs/Center for Forensic Science Research and Education, Willow Grove, PA 19090*

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**Learning Overview:** After attending this presentation, attendees will better understand how forensic and investigative science services are delivered in Brazil as well as the challenges faced by forensic scientists working in a developing country. Attendees will also gain an understanding of the differences between judicial perspectives on forensic science between Brazil and the United States and will better appreciate opportunities for collaborative scientific exchange.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the cultural and particular needs of Brazil's forensic and investigative science institutions, the challenges they face, and the opportunities for stronger working relationships, which would allow for growth of the field in both countries.

The 2019 American Academy of Forensic Sciences (AAFS) International Educational Outreach Program (IEOP) will visit Brazil to solidify the relationships that have been established with the mutual recognition between the Brazilian Academy of Forensic Sciences (BAFS) and the AAFS. Brazil is a country whose economy is ranked in the top ten largest in the world; however, its forensic and investigative services are still developing. Contemporary practice has made major strides toward advancement; however, Brazil still faces a number of challenges, including funding, awareness/education, and training that must be overcome to continue to improve best practices.

This session, designed to provide attendees with an orientation to the structure and delivery of forensic and investigative sciences in Brazil, is part of an ongoing initiative of the BAFS and the AAFS to recognize forensic science as a field without borders, providing a platform for international collaboration. The BAFS is the first association academy recognized by the AAFS. It was founded in 2012 with the goal of contributing to the development of forensic sciences in the country, guaranteeing justice and human rights.

In addition to examining the current state and organization of forensic science services in Brazil, Brazilian judicial perspectives on forensic science will be discussed. Ongoing and future opportunities for collaborations, such as scientist exchanges, Standard Operating Procedure (SOP) sharing, training prospects, and joint research collaborations, will be highlighted. Speaker presentations will conclude with an open question-and-answer session where attendees will be able to interact with the speakers to gain additional information.

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### Forensic Science, Brazil, Collaboration



### ES1 Adding Forensic Relevance to the Pediatric Autopsy With Defined Preautopsy Goals and Practical Techniques Using a Case-Based Approach

*Michael J. Caplan, MD\**, Suffolk County Office of the Medical Examiner, Hauppauge, NY 11788; *Amy T. Sheil, MD\**, Waukesha County Medical Examiner's Office, Waukesha, WI 53188

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**Learning Overview:** After attending this presentation, attendees will be able to: (1) describe the purpose of the autopsy in the evaluation of sudden unexplained or unexpected deaths in the setting of pediatric cardiac conditions (including therapeutic interventions and their complications); (2) highlight the added benefits of an autopsy when accompanied by ancillary studies in the evaluation of sudden unexplained deaths in infants and children with undiagnosed infectious diseases or metabolic or genetic disorders; (3) identify mimics of child maltreatment (abuse and neglect) revealed by postmortem examination; (4) describe and guide an approach to a pediatric autopsy in a death during or following (and potentially related to) a therapeutic procedure; (5) effectively communicate the value of the negative findings in a pediatric autopsy (“negative autopsy”) to clinicians and families; (6) improve their own institution’s autopsy practices through learning points gleaned by exposure to case-based examples; and (7) gain experience and proficiency with particular techniques relevant to the pediatric autopsy through use of a supplementary tutorial of specialized autopsy procedures and dissections.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving attitudes and practices regarding autopsies and by making the pediatric autopsy a more useful and serviceable tool for the consumers of the information imparted by the autopsy. The two primary goals of the presentation are to: (1) provide an initiative to enhance the practical value (diagnostic and educational yield) of a pediatric autopsy; and (2) apply the lessons gleaned from the autopsy to improve patient outcomes and reduce risks of recurrence of adverse outcomes.

Autopsy training in pathology residency varies greatly among teaching institutions, and resources including case material and faculty time are unevenly distributed. With diminishing numbers of hospital autopsies, including pediatric and perinatal autopsies, the opportunity for pediatric pathologists to gain proficiency and competence in performing autopsies has become more limited. Many pathologists in practice perform too few autopsies to maintain optimal skills and practices. Increasing reliance upon pathologists’ assistants to conduct gross examinations further restricts the experience of practicing pathologists. There exists a real risk among pediatric pathologists of insufficient ongoing experience with pediatric autopsies. There is also a tendency to include data in the body of the autopsy report that has limited clinical significance or that is incompletely addressed. All of these factors conspire to undermine the potential value and utility of the pediatric autopsy in clinical practice. These issues illustrate gaps in practice experience and current knowledge. There exists a definite need to provide education for practicing pediatric pathologists to enhance the clinical usefulness and relevance of autopsy reports. Forensic pathologists are in a potentially advantageous position to help pediatric pathologists with this challenge because, since autopsies are the mainstay of their primary activities, they understand the practical importance of delivering meaning and relevance to individuals to whom they need to present their findings in various medicolegal settings. The ultimate goal of this presentation is to facilitate communication and crosstalk between forensic and pediatric pathologists in order to achieve optimal benefits of pediatric autopsy results.

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#### Pediatric, Autopsy, Unexplained Death



### W1 What's Trauma and Stress Got To Do With It? Recognizing the Impact of Trauma on Forensic Professionals and Taking Steps To Mitigate It

*Amanda L. Farrell, PhD\*, Marymount University, Arlington, VA 22207; Lurena A. Huffman, BS\*, EMDC Forensics LLC, Hampton, VA 23666; Timothy J. Ainger, PhD\*, University of Kentucky, Lexington, KY 40536; Christopher J. Scallon, MS\*, Norfolk Police Department, Norfolk, VA 23510*

**Learning Overview:** After attending this session, attendees will understand and recognize how stress and trauma are tied to the forensic professions, including in the form of vicarious trauma, and how exposure to trauma and stress impacts the employee's personal and professional performance.<sup>1-7</sup> Attendees will be more aware of the various types of trauma that investigators and investigative personnel routinely encounter and the potential impacts this may have, not only on the individual, but on the investigation and/or service provision. Paths forward and the development of an Evidence-Based Policy (EBP) will also be addressed, so attendees can examine what policies and assistance are available in their own agencies or will be able to aid in policy development.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with the understanding that the human experience is variable, and trauma exposure and symptoms even more so, leading to behavior patterns that can be complex and abstract. By recognizing the manifestations of a variety of psychological—both behavioral and cognitive—impacts as a reaction to trauma and stress exposure on the job, as well as by identifying whether an agency is trauma-informed and what resources are available to employees, attendees will be better equipped to understand the short- and potentially short- and long-term impacts of trauma, as well as how to adapt investigative approaches and provide resources that will lead to increased chances of not only a positive work environment, but likely a more efficient and cost-effective solution to maintaining employee health and well-being to avoid burnout and decreased cognitive abilities that may impact service provision and job performance.

Cognitive implications after traumatic exposure are varied in presentation and require management but may be difficult for supervisors and forensic personnel to detect. Amnesic complications associated with dissociation, as well as shifts in cognitive schemas following traumatic exposure, have implications on both subsequent cognitive functioning and possible applications of insight-based treatment approaches.<sup>8,9</sup> The cognitive impact of trauma can be seen in both primary and vicarious/secondary trauma exposure (e.g., peers, treating clinicians).<sup>10</sup> Furthermore, some research has suggested a negative impact on executive functioning and memory, which may even be compounded by repeated exposure to trauma.<sup>11-14</sup> These cognitive skills, which are necessary for effective job performance for forensic professionals, are repeatedly being shown in the literature to be impacted by exposure to trauma.

Recognizing and addressing the impacts of direct and vicarious trauma on forensic personnel is especially important as employee burnout can represent not only the loss of an employee, but also a loss of the financial investment the agency or department has made in that individual's training and development, yet again highlighting the importance of striving for well-developed and empirically supported policies that protect both the financial interests of the agency and the jurisdiction, as well as the well-being of agency personnel. Even if the employee impacted by stress and trauma does not leave the agency, he or she may represent a financial loss to the agency in terms of decreased productivity, costs associated with workers' compensation, litigation fees, frequent absenteeism, and other similar costs.<sup>15-17</sup> In the current economic climate of budgetary cuts and a "do more with less" attitude, these statistics suggest that a cost-effective approach, and EBP should involve effective training and preparation, as well as swift intervention to avoid personnel developing long-term symptoms of psychological trauma and/or lingering cognitive deficits in the aftermath of trauma exposures. Thus, there are psychological, training, retention, and fiscal issues that cannot be disentangled from policy and practice and that the development of a comprehensive EBP is necessary.

Many times, professionals may not recognize the psychological impacts, behavioral and cognitive, of exposure or repeated exposure to trauma, and the easiest response may be to identify these impacts as an individual problem. However, as research, Critical Incident Stress Management Teams, Peer Support Teams, and the recognition of the impacts of vicarious trauma have demonstrated, these problems are more pervasive and of a greater magnitude than previously acknowledged in the first responder community. That recognition is slowly broadening to include investigative personnel and support personnel, as well. Based on previous research and work, this workshop provides a multidisciplinary approach that agency personnel, both line level and supervisory, can relate to and use to gain actionable insight.

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## Workshops – 2019

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### Forensic Professional Mental Health, Trauma, Cognition



### W2 “Will This Leave a Mark?” A Multidisciplinary Approach to Autoerotic Asphyxiation (AeA) Fatalities

Sharon L. Plotkin, MS\*, Miami Dade College, Miami, FL 33167; Teresa A. White, MA\*, Missoula, MT 59808; Tim Gallagher, MD\*, Pensacola Medical Examiner, Pensacola, FL 32504; Katherine Ramsland, PhD\*, DeSales University, Center Valley, PA 18034

**Learning Overview:** After attending this presentation, attendees will more comprehensively understand AeA deaths. This multidisciplinary approach will review the literature on circumstances preceding AeA deaths, discuss a practical approach to medicolegal death scene investigation, the certification of cause and manner of death, intentionality versus unintentionality AeA death case reviews, etiological factors, and psychological manifestations of practitioners, as well as stigmatic concerns families face over the loss of loved ones.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating techniques in AeA investigations. This is a multidisciplinary process involving medical and non-medical professionals for the correct certification of manner and cause of death in suspected AeA cases.

AeA is the practice of decreasing oxygen flow to the brain, via chemical or mechanical means, for the purpose of increasing the pleasure of a masturbatory orgasm. When the brain is deprived of oxygen, it induces a lucid, semi-hallucinogenic state called hypoxia. When combined with an orgasm, the euphoric state of hypoxia is compared to the effects of cocaine ingestion and can be highly addictive.

Erotic asphyxiation is a sexual practice variously called asphyxiophilia, autoerotic asphyxia, or hypoxyphilia. It includes the practice of intentionally restricting oxygen to the brain of a sexual partner. The term autoerotic asphyxiation is used when the act is performed by a person to themselves for heightened sexual self-gratification.

An AeA death scene is a rare and unique occurrence, and the materials at the scene can mislead the novice death scene investigator. It is also not uncommon for the family of the deceased individual to manipulate or alter the death scene to avoid possible social defamation associated with AeA. It is important for the death scene investigator to recognize an altered death scene and report that to the medical examiner.

The practitioner of AeA is most often revealed at the medical examiner’s office. Death occurs through a fault in the safety mechanism that the practitioner designed to restore normal blood flow to the brain after the sex act is completed.<sup>1</sup> Since a majority of AeA practitioners utilize a ligature around their neck to decrease oxygen flow to their brains, a failure in this safety mechanism resulting in death shares common elements with asphyxial suicides. This can lead to circumstances whereby the AeA death is incorrectly certified as a suicide.<sup>2</sup> Consequently, most of the epidemiological knowledge of AeA comes from the practitioner who dies because of the act. The intention of the participant is not for a fatal outcome but to achieve a heightened sense of sexual gratification. Insurance companies have successfully argued that AeA is a high-risk act of bravura and therefore beneficiaries should not be granted financial recovery in these cases.<sup>3</sup> This type of argument discusses the intentionality of the act leading to death versus the unintentionality of death. Although AeA death is most consistently certified as an accident, the argument persists as to whether the practitioner could reasonably expect death to be a result of the act.

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#### Autoerotic Asphyxiation (AeA) Death, Equivocal Death, Death Investigators



### W3 Child Murder by Parents: Psychiatry, Pediatrics, and Pathology

*Carl Wigren, MD\**, Wigren Forensic PLLC, Seattle, WA 98101; *Susan Hatters-Friedman, MD\**, University of Auckland, Auckland, NEW ZEALAND; *Renee Sorrentino, MD\**, Institute for Sexual Wellness, Weymouth, MA 02188; *Josh Friedman, MD, PhD\**, Cleveland, OH 44106; *J. Paul Fedoroff, MD*, Royal Ottawa Hospital, Ottawa, ON K1Z 7K4, CANADA

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**Learning Overview:** After attending this presentation, attendees will understand child murder by parent cases from psychiatric, pediatric, and pathology perspectives. The discussion will include an understanding of parental motives and common factors behind child murder; diligently searching for the explanation and mechanism of the suspected inflicted injury; and courtroom testimony when understanding biases. The panel will include forensic psychiatrists (including perinatal psychiatry subspecialty), a child protection pediatrician, and a forensic pathologist. Case examples will be provided.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing competence in understanding the phenomenon of child murder by parents, effectively evaluating alleged perpetrators and the medical evaluation of such cases. This should lead to more scientific and less biased evaluations.

*Diligence, Dedication, and Devotion* are critical in evaluations of child murder by parents. Child murder by parents is often mischaracterized by well-meaning psychiatrists and jurists due to flawed interpretations of the literature. In two dozen nations, child murder by mothers has its own charge or defense of infanticide when the child killed is under a certain age. Those mothers may have no sentence or a sentence akin to that for manslaughter. Nothing comparable exists for fathers who kill. Yet mothers who make use of the defense are often not suffering from severe mental illness. Mothers who kill their children are often met with sympathy while fathers are vilified. Deservedness is much more nuanced. Research findings from both the psychiatric and the criminological studies will be discussed to further understand the characteristics of these parents. Conducting appropriate non-gender-biased insanity evaluations and mitigation evaluations in such cases will be reviewed. Fatal inflicted pediatric trauma in some cases are hospitalized prior to their passing and involve child protection pediatricians. Relevant knowledge and experience from child protection pediatrics will be presented. On the death of the child from suspected inflicted injury, forensic pathologists and medical examiners work to determine cause of death. With available information often incomplete, child abuse pediatricians and forensic pathologists provide opinions regarding plausible mechanisms of injury. This has potential impacts on the criminal court case as well as for custody.

Cases will be discussed that demonstrate these themes, with audience participation. A question-and-answer session at the end of the workshop will enable attendees to ask questions of the speakers. The goal of the panel is to educate attendees on the phenomenon of child murder by parents from the perspectives of forensic psychiatry, pediatrics, and pathology.

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#### Child Murder, Filicide, Infanticide



### W4 The Medicolegal Investigation of Recreational Diving Fatalities

*James Louis Caruso, MD\*, Office of the Medical Examiner, Denver, CO 80204; Michael D. Bell, MD\*, Palm Beach County Medical Examiner's Office, West Palm Beach, FL 33406*

**Learning Overview:** After attending this presentation, attendees will: (1) have a basic understanding of the special physiology and specialized equipment associated with scuba diving; (2) appreciate the epidemiology of deaths associated with recreational diving, including geographic distribution, common causes of death, and contributing factors to these fatalities; (3) be able to adequately investigate and interpret the historical events and circumstantial evidence associated with diving fatalities; (4) understand the recommended approach to the autopsy of persons who died while diving and be able to interpret the anatomical findings in the context of the historical events; and (5) have handout material and points of contact for future reference to competently investigate a diving-related death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the training and resources needed to forensic pathologists and medicolegal death investigators, so they may competently and confidently investigate recreational (scuba) diving-related deaths.

The popularity of recreational diving using Self-Contained Underwater Breathing Apparatus (SCUBA) has increased dramatically over the past several decades. Present estimates place the number of active recreational divers in the United States at between 500,000 to 1,000,000. The number of fatalities involving United States citizens performing recreational dives averages 80 to 90 each year. These fatalities challenge the investigators and pathologists who must investigate and certify these deaths. Recreational diving fatalities are often litigated in civil court. This workshop is designed for the pathologist, criminalist, attorney, and general section member who may become involved in the investigation of a scuba diving accident or fatality.

The initial portion of this workshop will include a brief overview of diving physiology, including the effects on the body of breathing compressed air. The pathophysiology of barotrauma, nitrogen narcosis, oxygen toxicity, gas embolism, and decompression illness (caisson disease) will be reviewed. The pathophysiology of drowning will be reviewed, since it is a frequent outcome in a fatal diving mishap.

Following the discussion on physiology, the epidemiology and risk factors associated with recreational diving fatalities will be presented. A detailed presentation on the recommended investigation of a fatal diving mishap will be given using illustrative cases from several jurisdictions. The importance of interviewing witnesses and gathering information on the diver's past medical history, diving experience, pre-dive status, and the circumstances surrounding the dive will be emphasized. The relevance of knowing the exact depth and bottom time of the dive, as well as when and where the diver began to run into difficulty, will be discussed. Additionally, this workshop will include a hands-on section in which typical diving equipment will be available for examination and familiarization by attendees. This will be accompanied by a brief discussion on the evaluation of dive gear.

In the final portion of the workshop, the speakers will review the autopsy protocol for scuba diving victims and emphasize those tests and observations that are helpful in determining the cause of death. The significance of finding intravascular bubbles will be discussed, as will the proper interpretation of the findings of the autopsy. Natural diseases that are likely to cause sudden incapacitation and death while scuba diving will be reviewed. Finally, related topics, such as hazardous marine animals, zoophagia, and trauma leading to recreational diving fatalities, will be presented.

Thorough handouts will be provided by the speakers, including checklists of important information to obtain regarding a diving mishap, a diving fatality reporting form, a suggested autopsy protocol for use on a diving related fatality, and recommended resources for consultation and referral.

#### SCUBA Diving, Drowning, Air Embolism



### W5 Novel Psychoactive Substance (NPS) Surveillance, Detection, and Intelligence for Use by Forensic Laboratories, Public Health, and Public Safety

*Alex J. Krotulski, MS\**, Center for Forensic Science Research & Education, Willow Grove, PA 19090; *Barry K. Logan, PhD\**, NMS Labs/Center for Forensic Science Research & Education, Willow Grove, PA 19090; *Jason A. Bordelon\**, Drug Enforcement Agency, Springfield, VA 22152; *Francis X. Diamond, BS\**, Willow Grove, PA 19090; *Donna M. Papsun, MS\**, NMS Labs, Willow Grove, PA 19030; *Andrew Stolbach, MD\**, The Johns Hopkins University Medical School, Baltimore, MD 21205; *Rudolph C. Johnson, PhD\**, Center for Disease Control and Prevention, Atlanta, GA 30341; *Eric D. Wish, PhD\**, Center for Substance Abuse Research, Colledge Park, MD 20740-3210; *Jae W. Chung, BS\**, Drug Enforcement Agency, Springfield, VA 22152; *Jeffrey Lai, MD\**, University of Massachusetts Medical School, Department of Emergency Medicine, Worcester, MA 01655; *Elizabeth Young Laanemets, MSc*, Canada Border Services Agency, Ottawa, ON, CANADA K1A 0L8; *Jason M. Piotrowski, BA\**, New Jersey Regional Operations & Intelligence Center, West Trenton, NJ 08628; *M.J. Menendez, JD\**, Organized Crime Drug Enforcement Task Force, Washington, DC 20530;

**Learning Overview:** After attending this presentation, attendees will be able to: (1) understand approaches for the surveillance and initial detection of NPS; (2) describe methods for the analysis of case evidence for characterization of emerging or unknown NPS; (3) assess analytical and clinical findings of NPS in casework and intoxications; and (4) demonstrate manners for which data can be used to track or monitor trends and overall NPS intelligence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing real-time information on NPS detection and emergence, the opioid epidemic, clinical treatment strategies, and large data dissemination tactics. Presentations will cover a variety of NPS classes, with specific focus on fentanyl analogues and novel opioids, detected and identified in recent casework. Additionally, the presentations in this workshop will showcase the multidisciplinary efforts being conducted to detect NPS and report their presence in seized materials or biological fluids.

Identification and characterization of NPS has become critical among forensic, clinical, public health, and public safety communities. Proliferation of new drugs and illicit substances has increased the challenges associated with typical or routine testing procedures, law enforcement efforts, and treatment workflows. Most notably, the current expansion of novel opioids in the street drug supply, consisting of a vast variety of fentanyl analogues and U-series derivatives, has resulted in a skyrocketing number of adverse events, including death, in recent years. It has become clear that combating the opioid epidemic and NPS proliferations are not singularly on one scientific field or government agency, but rather a multidisciplinary effort among law enforcement, forensic scientists, clinicians, public health officials, and other pertinent communities. Interdiction and intervention are not possible without full support and commitment from all agencies and communities, as this has been demonstrated by organizations showcased during this workshop. Detection and characterization of newly emerging substances is key to remaining at the forefront of this ever-evolving issue. Identifications fuel intelligence efforts for monitoring trend information, developing emergence data, and notifying respective communities. Notifications allow for surveillance of NPS and novel opioids through tracking of shipments, migration of drug supplies, and monitoring of new or suspicious activities. Surveillance leads to the acquisition of packages, drug supplies, and/or biological fluids that feed into laboratories for analysis. All three of these aspects work in unison and create a cycle of information sharing.

This workshop will provide information related to the sale and acquisition of NPS, the migration of NPS to the point of initial identification and subsequent detections, and the end use of the information for intelligence or surveillance purposes. Analytical methods and tools for NPS detection in forensic chemistry and forensic toxicology will be discussed, in addition to timely results from analysis of casework specimens, including seized materials and biological fluids. Additional topics included in the program will focus on clinical approaches to the identification of NPS and appropriate dissemination of results, as well as methodologies for the compilation and distribution of data and findings, specially relating to large population sets with all-encompassing information.

#### Novel Psychoactive Substances, Novel Opioids, Surveillance



### W6 The Impact of Emerging Technologies on Digital & Multimedia Forensics

Steven B. Watson, BA\*, VTO, Broomfield, CO 80021-8042; Marla E. Carroll, BS, Forensic Video & Audio Associates, Inc, Plantation, FL 33317; Jeff M. Smith, MS\*, National Center for Media Forensics - CU Denver, Denver, CO 80204; Mary F. Horvath, MFS\*, Ft. Myers, FL 33966; James Darnell, BS\*, United States Secret Service, Tulsa, OK 74104; Matthew Domanic, BA\*, VTO, Inc, Broomfield, CO 80021; Boyd Hamilton\*, Fidelis K9, Tulsa, OK 74104; Jessica Hyde, MS\*, Magnet Forensics, Herndon, VA 20171

**Learning Overview:** The goals of this presentation are to: (1) inform participants of emerging technologies affecting digital forensic science today; (2) identify technologies on the horizon that will be impacting the world of forensics science; (3) learn practical techniques for addressing new technology questions in their field of practice; (4) network with other interested researchers, academics, and practitioners to affect their thinking on new and emerging technology issues; and (5) learn of real-world implications as case studies are presented across a wide variety of topics throughout the workshop.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of current emerging and horizon technologies impacting the world of forensic science today. This presentation will stimulate research among the participants and their organizations and provide opportunity for participants of varying interests to identify like-minded individuals to collaborate on research ideas. Finally, this presentation will continue to establish the American Academy of Forensic Sciences as a leader in the world of digital forensic science through forward-looking preparation of the digital forensics community for the challenges that are coming.

As technology continues to evolve and morph into new formats and platforms, the challenge of digital and multimedia forensics progresses as well. Digital forensics experiences dynamic changes caused by the rapid progression of technology, new inventions with new applications, and the continued miniaturization and complexity of the technologies arriving at digital forensics labs. Looking toward the horizon, bigger challenges emerge with how scientists will successfully perform digital forensics on new platforms that do not yet exist. New methods for storing data are emerging with ways that computing can be performed across new types of devices that have not yet been encountered in the history of digital forensics.

While many forensic science disciplines encounter iterative change enabled by new methods and new technologies, digital forensic science encounters revolutionary change across devices and technologies not yet created. Digital forensic practitioners are heavily affected by the market forces driving adoption of new technologies. Market adoption of emerging technologies leads to these same new technology devices appearing in evidence queues to be addressed by practitioners that have not yet been trained on these devices. What methods and research exist in emerging technology areas that can inform discreet problem statements while finding applicability across other new technology devices as well?

Garfinkel identified that standardization and validation must be strengthened to meet the challenge coming with future technologies.<sup>1</sup> Lillis et al. identify that future digital challenges in and of themselves can hamper investigations, but the cumulative effect amplifies these difficulties.<sup>2</sup> Caviglione et al. call out the need for multidisciplinary approaches spanning multiple fields to address the future of digital forensics.<sup>3</sup>

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3. L. Caviglione, S. Wendzel, and W. Mazurczyk. The Future of Digital Forensics: Challenges and the Road Ahead. *IEEE Security & Privacy*. Vol. 15, no. 6, pp. 12-17, 2017. doi:10.1109/MSP.2017.4251117.

#### Digital Forensics, Emerging Technologies, Future Technologies



### W7 A Multidisciplinary Approach for Cardiovascular Deaths: Breakthrough Technologies and Their Applications

*Katarzyna Michaud, MD\**, Centre Universitaire Romand de, Lausanne 1000, SWITZERLAND; *Silke Grabherr, PhD\**, Centre Universitaire Romand de Médecine Légale, Lausanne 25, Vaud 1000, SWITZERLAND; *Sara Sabatasso, MD\**, Geneva 1211, SWITZERLAND; *Stephen D. Cohle, MD\**, Spectrum Health-Blodgett, Grand Rapids, MI 49506; *Morgane Jotterand, MD\**, University Center of Legal Medicine, Lausanne, SWITZERLAND

**Learning Overview:** After attending this presentation, attendees will better understand the use of modern technologies by immunohistochemistry, metabolomics, genetics, and imaging introduced recently in postmortem evaluation of cardiovascular pathologies, not only for natural cases but also for traumatic and post-cardiac surgery cases involving medical liability. Participants will understand the advantages and limitations as well the possible pitfalls of these methods.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by presenting new technologies (immunochemistry, genetics, and imaging) and their current role and prospective. By including discussion with participants, the usefulness of these methods will be clearer to the forensic science community.

Any sudden, unexpected or unexplained death, mainly in the young, is a reason for a medicolegal investigation. The underlying cause of sudden death is most frequently cardiovascular with coronary atherosclerotic disease as the leading cause of death in elderly victims, while genetically determined cardiomyopathies as hypertrophic or arrhythmogenic cardiomyopathy and canalopathies prevail in the young. Modern immunohisto-chemical markers and untargeted metabolomics allow the progress in the detection of early myocardial ischemia. Autopsy-negative Sudden Cardiac Deaths (SCDs) are most often thought to be the consequence of sudden arrhythmic death syndrome, and so-called molecular autopsy is recommended. Radiological examination has been used in forensic and clinical pathology in specific situations of natural and violent deaths for many years, as an additional diagnostic tool, for documentation and for research, and is very useful for cardiovascular pathologies.

Many immunohistochemical markers have been proposed to diagnose early myocardial infarction, which include proteins from the complement cascade (C5b9), fibrinogen and fibronectin, and some of them seem promising in terms of early expression and specificity. Untargeted Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI IMS) imaging was also evaluated. These methods need to be tested and validated for application in routine postmortem diagnosis. The interpretation of immunohistochemical staining should consider cardiopulmonary resuscitations and other agonal events that may affect marker expression. Current insight is that immune stains cannot reliably detect myocardial ischemia at an earlier time point than conventional staining. However, they can be valuable for the confirmation of diagnosis and demonstration of the extent of myocardial necrosis in the heart.

The potential genetic origin of cardiac pathologies involves the forensic pathologist in the multidisciplinary management of surviving family members. The role of the forensic pathologist is to perform and store the postmortem samples according to legal and ethical guidelines and orients the relatives of SCD's victims to the genetic counselling. Most of the laboratories today use Next Generation Sequencing (NGS), and it is possible to screen several genes at the same time, but the interpretation of the results requires a close collaboration with clinicians.

In forensic investigations, a significant progress has been made with the introduction of modern radiological methods, such as Multiple Detector Computed Tomography (MDCT), MDCT-angiography, and cardiac Magnetic Resonance Imaging (MRI). These methods are also practiced for cardiovascular pathologies, mostly ischemic heart disease. The advantages and disadvantages of various radiological methods for whole-body imaging are related to logistic and financial questions, as well as those concerning the image quality. The training necessary to interpret postmortem radiological images is a subject of discussion. The question concerning the value of minimally invasive autopsy techniques has appeared.

In sudden cardiac death cases, Postmortem Computed Tomography (PMCT) is helpful to estimate the heart size and to visualize hemopericardium, calcified plaques and valves, as well as to identify and locate cardiovascular devices. Angiographic methods are useful to provide a detailed view of the coronary arteries and to analyze them, especially regarding the extent and location of stenosis and obstruction. In post-surgical cases, it allows verifying and document patency of stents and bypassing grafts before opening of the body. Postmortem Magnetic Resonance Imaging (PMMRI) is available in only a few academic centers and is practiced to investigate the soft tissues, such as the myocardium; Postmortem Magnetic Resonance-angiography (PMMR-angiography) is today in development; detection of myocardial ischemic injury in PMMRI has been reported, but images are susceptible to being affected by postmortem changes, and further work is ongoing in order to increase understanding of radiological aspects of the ischemic myocardium. In violent and post-surgery cases, the value of postmortem imaging of the heart is reported essentially for diagnostic and documentation purposes. It is recognized that postmortem radiological examination is most often not sufficient to establish the exact cause of cardiac death and, at present, autopsy remains the gold standard.

#### Postmortem Radiology, Sudden Cardiac Death, Postmortem Genetic Testing



### W8 A Decade Later: *The State of Florida v. Casey Marie Anthony* Revisited—Truth? Justice? Both? Neither?

*James J.C.U. Downs, MD\*, forensX, LLC, Savannah, GA 31406; Anjali A. Ranadive, JD\*, SciLawForensics, Ltd, Brookings, SD 57006; Linda Kenney Baden, JD\*, New York, NY 10019; Bruce A. Goldberger, PhD\*, University of Florida College of Medicine, Gainesville, FL 32608; Neal H. Haskell, PhD\*, Rensselaer, IN 47978; Henry C. Lee, PhD\*, University of New Haven, West Haven, CT 06516; Barry K. Logan, PhD\*, NMS Labs/Center of Forensic Science Research and Education, Willow Grove, PA 19090; Haskell M. Pitluck, JD\*, Crystal Lake, IL 60014; Steven A. Symes, PhD\*, Mississippi Medical Examiner's Office, MCL, Pearl, MS 39208; Jan C. Garavaglia, MD\*, Burlington, WA 98233; Anne E. Perez, PhD, Ohio University, Department of Biological Sciences, Athens, OH 45701*

**Learning Overview:** After attending this presentation, attendees will be better able to consider differing interpretations of data specific to the presented case as well as apply principles to the broader arena of the forensic sciences in general. The goal of this presentation is to help individuals to better understand the forensic practitioner's role in evaluating the evidence and the propriety of the outcome and to evaluate the analyst's role in the overall totality of the case. Ultimately, the forensic analyst, regardless of discipline, should understand his/her duty to the truth and objectivity above all else.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with the knowledge to allow them to better understand and articulate the nature of their role in forensic cases and to apply that comprehension, whatever the overall outcome of a case. In addition, attendees will be better positioned to effect positive change to ensure that impartial, objective scientific and medical analyses are employed in casework and testimony.

**July 15, 2008:** "The happiest place on earth." A decade ago, the case that would captivate a country begins with a simple phone call. A woman contacts the local police to report her granddaughter had been missing for a month.

**October 14, 2008:** The girl's mother is indicted for first-degree murder, even though, to that point, no body has been found.

**December 11, 2008:** The badly decomposed body is found in a wooded bog just down the street from the family's home. After extensive forensic work and investigation, with the homicide determined to be due to "undetermined means," the venue moves to the courtroom for the death penalty case two years later, in May 2011.

Under the glare of television lights, the high-profile trial of the day began in earnest. The recent explosion of social media changed forever the nature of such cases. With the surging popularity of infotainment, the public followed the case—many with minds fully decided as to guilt or innocence of the accused (who came to be known as "the most hated woman in America") prior to opening statements or even the first witness being heard. The public literally took to the streets to stand in line for a chance at courtroom seats. The diametrically opposed versions of events played to a packed house in what became a theater of the courtroom.

The indisputable facts of the case remain clear and simple: a 2-year-old toddler disappeared and her then-22-year-old single mother never reported her missing. The mother subsequently lied to investigators, inventing a non-existent theme park job, a "Zanny the nanny," and a conversation with the child (the very day she was reported missing).

The state presented extensive investigative and forensic evidence. The defense countered with allegations of unproven science and bias. The focus quickly shifted to a battle of experts. The defense suggested alternate theories to account for some of the findings. Tensions between the sides ran so high that the judge ordered that neither side could disparage the other and had to admonish counsel during closing arguments. After a six-week trial, the mother was acquitted of murder but convicted on four misdemeanor counts.

This session will examine forensic and legal aspects of this contentious trial and verdict. The multidisciplinary analysis includes critical players from the actual trial and a distinguished group of other experts. Disciplines covered include forensic pathology, anthropology, toxicology, entomology, criminalistics, and jurisprudence. Experts are expected to occasionally have widely varied perspectives and opinions regarding case materials and analyses; however, differences in professional opinions should not be clouded by unrelated issues. In an effort to seek a fuller, objective understanding of the case and what happened, the speakers will attempt to distinguish fact from allegation, insinuation, suspicion, etc. An important issue in this case is defining the line separating accepted forensic expertise from mere opinion and understanding that such a line may not, in fact, be clear. An often underappreciated codicil is: "Who is best qualified to establish such a line?"

With the blurred edge between fact and supposition and with the polarization of opinions regarding this case and its outcome, it is hardly surprising that this tragic case can still serve as a valuable example for the forensic practitioner. A decade later, interest in the case remains high—as does emotions regarding the case. Some bemoan what they describe as a "fantasy of forensics" and a "free-for-all of forensics" targeted at convicting the accused. Others wonder how on earth an abundance of compelling evidence of guilt could have resulted in the verdict. The distinguished seminar faculty will present divergent views of the facts of the case and their interpretation. In the end, only the jurors know the answer to the questions of "Why?" and, ultimately, "Was justice served?"

#### Casey Anthony, Objectivity, Decomposition





### W9 Deciphering Complex Electrostatic Detection Device (EDD) Impressions

*Mark Goff, BA\*, MSP Lansing Laboratory, Lansing, MI 48913; Jennifer Furman, MFS, Springfield, VA 22152*

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**Learning Overview:** The goals of this workshop are to provide attendees with the knowledge, skills, and abilities to create layered images for the decipherment of complex impressions developed by EDD using commonly available digital imaging software. These skills will be demonstrated on multiple and widely varied examples of real-world problems that benefit from this method.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a methodology that will increase the accuracy of interpretation of impressions developed by EDD and a reduction in the cost of these examinations in material and time.

Examining EDD-developed impressions with digital image layers expands on previous methods of tracking sourced impressions and imaging techniques. Early techniques of removing interfering images from EDD lifts, such as embossed writing or impressions sourced to available writing, required complex photography to subtract these interfering impressions. A subsequent technique involved the creation of multiple transparencies to create overlays. These transparencies were then stacked on top of each other to account for sourced impressions and interfering embossed writing. While both methods are effective, they can be resource and labor intensive.

This workshop will teach a modern approach to the transparency method for tracking sourced impressions using Adobe® Photoshop® layers. This workshop will demonstrate how creating layer masks of available writing and common sourced impressions can be used to account for impressions from multiple documents. This method can also be used to demonstrate the orientation of the impressions when created, reveal unsourced impressions in documents containing complex impressions, and associate documents containing impressions from a common source.

Practical applications of this method include: subtracting impressions of visible writing from preceding pages and following pages of sequential documents to account for unsourced impressions from missing pages; differentiating embossed writing from writing impressions; and separating “layers” of complex impressions in the same document. Additionally, this method can also be very helpful when trying to determine if impressions were created contemporaneously. The final layered image creates a powerful interactive demonstrative product for court testimony.

As a skills workshop, it is paced to maximize the amount of time spent working with imaging software using hands-on examples to ensure these techniques can be used when the attendee returns to the work site.

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#### Documents, EDD, Impressions



### W10 DNA Mixture Interpretation Principles: Observations From a National Institute of Standards and Technology (NIST) Scientific Foundation Review

*John M. Butler, PhD\**, NIST, Gaithersburg, MD 20899; *Sheila Willis, PhD\**, NIST, Gaithersburg, MD 20899; *Melissa K. Taylor, MA\**, Gaithersburg, MD 20899; *Rich Press, MSc\**, NIST, Gaithersburg, MD 20899; *Hariharan Iyer, PhD\**, Gaithersburg, MD; *Peter M. Vallone, PhD\**, NIST, Gaithersburg, MD 20899-8314; *Bruce J. Heidebrecht\**, Maryland State Police, Forensic Sciences Division, Pikesville, MD 21208; *Jennifer Gombos Breaux, MFS\**, Clarksburg, MD 20871; *Eugene Y. Lien, MS\**, New York City Office of Chief Medical Examiner, New York, NY 10016; *Keith Inman, MCrim\**, Department of Criminal Justice Administration, Hayward, CA 94542; *Roger Frappier, MSc\**, The Centre of Forensic Sciences, Toronto, ON M3M 0B1, CANADA; *Charlotte J. Word, PhD\**, North Chesterfield, VA 23235-0153; *Lisa C. Schiermeier-Wood, MS\**, VA Dept of Forensic Science, Richmond, VA 23219; *Joel D. Sutton, MSFS\**, United States Army Criminal Investigation Laboratory, Forest Park, GA 30294; *Robin W. Cotton, PhD\**, Boston University School of Medicine, Boston, MA 02118; *Jack Ballantyne, PhD\**, University of Central Florida Department of Chemistry, Orlando, FL 32816-2366; *Ray Wickenheiser, MBA\**, New York State Police Crime Laboratory System, Albany, NY 12226-3000

**Learning Overview:** After attending this presentation, participants will better understand the principles involved with DNA mixture interpretation, knowledge of core foundational literature supporting these principles, and approaches to establishing interpretation guidelines for DNA mixtures, including approaches that involve probabilistic genotyping software.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the importance of having documented research and validation studies to support measurement and interpretation claims in forensic science.

DNA mixture interpretation has become more challenging in recent years due to several factors, including submission of more touch evidence samples to aid investigations and the generation of more sensitive DNA test results with new Short Tandem Repeat (STR) typing kits. This sensitivity enables recovery of DNA results from low quantities of biological material. However, the Polymerase Chain Reaction (PCR) signal amplification process, which provides the DNA test sensitivity, also introduces artifacts into the resulting data, such as stutter products, as well as ambiguity in the form of allele drop-out, allele drop-in, and heterozygote imbalance that occur due to stochastic effects. These artifacts, combined with allele sharing among contributors to the mixture, make pairing contributor alleles into their donor genotypes more difficult and increase uncertainty associated with the mixture interpretation process. Probabilistic genotyping software programs are being implemented in many laboratories to aid evaluation of low-template DNA results, either from single-source samples or trace components of mixtures.

The NIST has been congressionally-funded to perform scientific foundation reviews of select forensic disciplines. These reviews are intended to establish what is well-known and well-supported empirically in a forensic field and identify gaps that need further study. DNA mixture interpretation was selected as the initial NIST scientific foundation review, given the existence of abundant literature and a need expressed by members of the community. Multiple interlaboratory studies conducted by the NIST and others have noted variability among accredited laboratories using validated approaches on the same DNA mixture data.

Since September 2017, a NIST review team has been studying issues surrounding DNA mixture interpretation. An important goal of this project is to identify, consolidate, and share core principles and supporting publications with the community to encourage deeper learning and understanding of DNA mixture interpretation. More than 500 articles related to DNA mixture interpretation have been gathered and examined to better understand capabilities and limitations as reflected in the scientific literature. An external DNA Mixture Resource Group, composed of 13 experienced practitioners and technical leaders, has provided valuable input and feedback to the NIST team on a regular basis. Findings from this study will be shared, along with primary considerations identified.

The report from this study, entitled *DNA Mixture Interpretation: A NIST Scientific Foundation Review*, is in the process of being written to cover a variety of topics in seven chapters. Chapter 1 introduces the need and approach to the study. Chapter 2 provides historical perspective and reviews current methods for DNA mixture interpretation. Chapter 3 discusses the NIST review team efforts and input received from outside experts along with data sources and literature evaluated. Chapter 4 covers case context and relevance issues important to high sensitivity methods where DNA transfer and persistence are possible. Chapter 5 examines measurement uncertainty issues and notes some approaches to helping validation studies become more performance-based rather than task-driven. Chapter 6 explores capabilities and limitations of new technologies, along with forces at play that influence acceptance and adoption of new approaches, and touches on training challenges and needs going forward. Chapter 7 summarizes key takeaways and considerations to help the field move forward. The report Appendix contains an in-depth reference list with annotated foundational articles.

#### DNA, DNA Mixtures, DNA Evidence Interpretation



### W11 Interpreting Blunt Force Injuries in Bone: A Hands-On Approach

*Steven A. Symes, PhD\*, MS Medical Examiner's Office, MCL, Pearl, MS 39208; Ericka N. L'Abbe, PhD\*, University of Pretoria, Pretoria 0001, SOUTH AFRICA; Mark M. LeVaughn, MD\*, Office of the Chief Medical Examiner, Pearl, MS 39208; Anastasia Holobinko, PhD\*, Mississippi State Medical Examiner's Office, Pearl, MS 39208; Cortney N. Hulse, MA\*, Reno, NV 89503; James J.C.U. Downs, MD\*, forensX, LLC, Savannah, GA 31406*

**Learning Overview:** After attending this presentation, attendees will have: (1) participated in several hands-on analyses of blunt force injuries of processed cranial and postcranial remains; (2) received peer and facilitator feedback on their analyses and interpretations; (3) acquired knowledge from a forensic anthropologist and pathologist on how fracture biomechanics methodology is used to interpret and validate fracture patterns and total body trauma in a medicolegal environment; and (4) experienced common errors in analysis and interpretation of broken bones.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with hands-on experience in analyzing blunt force injuries. Mentored, real-life case experiences are necessary for practitioners to develop expertise within their discipline. Informal, or formal, mentorship is crucial for novices to develop the schema and attitudes necessary for expertise development and self-regulated learning. Practitioners who encountered constraints in their work environment, such as a lack of skeletal resources and/or role models, express disappointment in their work, are less skilled, and are less confident than their mentored peers. In this workshop, current theoretical knowledge will be applied by practitioners and facilitators to assist attendees in using this knowledge in describing and interpreting bone fractures within a medicolegal investigation.

Anthropologists and pathologists are increasingly requested to analyze and interpret bone trauma in a medicolegal setting, but many professionals remain hesitant, ineffective, or unable to adequately address skeletal trauma. Blunt injuries are the most common in a medicolegal setting, with many practitioners receiving little diagnostic training. The absence of the proper utilization of skeletal trauma analysis may be attributed to a lack of understanding of bone biomechanics and exposure to casework and, in many institutions, an absence of adequate reference material from which to learn. Knowledge of basic biomechanics and fracture pattern recognition, with some exceptions, is key to the accurate interpretation of trauma in the human skeleton. Many anthropologists and pathologists learn theoretical bone trauma principles as well as research biomechanics and traumatic injury to bone. However, few practitioners are exposed to the cases in which this theory needs to be accurately applied and in which not all factors involved in producing the injury are known or can be identified.

The approach that allows for the interpretation of fractures lies in the biomechanical response of bone to an injury. "Biomechanical response" refers to the bone's reaction to stress/strain. Under stress, a bone bends elastically and, if the stress dissipates, the bone will return to its original shape. However, if the stress continues and a fracture occurs as expressed in plastic deformation, then the bone will consequently fail. Fracture characteristics, such as compression, tension, and shear, can be used to infer particular features of the force. These are the speed of the force, its duration, its size and strength, and the direction of the force. Simply put, assessing biomechanical forces generates far more interpretable data than "the bone was 'pushed in' here." Bending bone is the root cause of the majority, if not of all, fractures. Therefore, the biomechanical results are predictable, interpretable, and invaluable for forensic anthropologists and pathologists.

#### Blunt Force Injuries, Osteology, Total Body Trauma Pattern



### W12 Voluntary Consensus Standards—Where They Come From and What They Mean for You

Lucy A. Davis, BHS\*, LDH Consultants, LLC, Pikeville, KY 41501; Sharon K. Stanford, BA\*, American Dental Association, Homewood, IL 60430; Teresa Ambrosius, BA\*, AAFS, Colorado Springs, CO 80904; Daniel G. Smith, MA\*, ASTM International, Hatboro, PA 19040; Michael Wixted, MA\*, National Fire Protection Association, Quincy, MD 02169; Jennifer F. Limoges, MS\*, New York State Police, Albany, NY 12226-3000; Kenneth W. Aschheim, DDS\*, New York, NY 10065; Kris C. Cano, MA\*, City of Scottsdale Police Department, Scottsdale, AZ 85257; Christine Funk, JD\*, Saint Paul, MN 55102; Vincent J. Desiderio, Jr., MS\*, United States Postal Inspection Service, Washington, DC 20260

**Learning Overview:** The goals of this presentation are to educate attendees on the process and products of different forensic Standards Developing Organizations (SDO) and provide practical examples from experts on the application of consensus standards to different forensic disciplines.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing education regarding the development of consensus-based standards and the emerging role those standards may have as laboratories begin to implement them.

The 2009 National Academy of Sciences Report on the status of forensics in the United States highlighted private sector standards as fundamental keys to a more effective forensic foundation across the country.<sup>1</sup> The Report emphasized the need for standards to shore up gaps in communications among field practitioners, testing laboratories, crime investigators, and the courts. The Organization of Scientific Area Committees for Forensic Science (OSAC) was formed to facilitate the development of technically sound forensic science standards and promote the adoption of those standards by the forensic science community. Many of the documents developed by OSAC committees are submitted to an SDO to undergo their accredited procedures for public comment and due process.

Voluntary Consensus Standards can seem like scary creatures. Rumors are flying regarding how they are developed in back alleys and that forensic scientists will be shackled with them and their evil ways for eternity. This workshop is designed to dispel those myths and rumors and promote peace and understanding between the forensic and standards worlds. Four SDOs that have published standards related to the forensic science will present information concerning standards and their organizations' processes. The American Dental Association (ADA) will provide an overall view of the nature of standards as a language that ensures product consistency and compatibility, allows for equitable competition, technology dissemination, and public welfare across multiple disciplines internationally, including how to include the perspectives and needs of all stakeholders. The AAFS Academy Standards Board (ASB) will discuss their standards development process and provide an overview of their operations and committee structure and will share examples of how of their standards and best practices are used in the forensic community. The American Society for Testing and Materials (ASTM) International will provide an overview of their Committee E30, their standards development process, and how resulting standards are used. The National Fire Protection Association (NFPA) will also present their processes and standards. After a question-and-answer period with the SDO representatives, forensic science practitioners will discuss their experience with standards within their disciplines. AAFS members who deal with toxicology, odontology, DNA, and trace material will provide practical examples of how standards are, or may be, applied to their professional practices in forensics. Also, a lawyer will present her prospective of standards and the application of them from the jurisprudence perspective. Another discussion period will be held with this panel.

The forensic science community should understand what consensus-based standards are available, how to apply them to real-time forensic practices, and adapt their methods, technology, and terminology to adopt the standards as they become available. The standards being developed will influence the future perception of forensic science applications and may become the basis for legal acceptance, accreditation, and certification programs. By individual forensic scientists understanding the standards development process and finding ways they can become actively involved in that process, they may better support and facilitate the adoption of the standards in their everyday work procedures.

Presentations and remarks of the panelists will enable attendees to understand the changing forensic landscape marked by the introduction and application of consensus-based forensic standards and development process. These presentations will present current and potential activities related to the development and application of consensus standards for forensic uses.

#### Reference(s):

- <sup>1.</sup> National Research Council. 2009. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/12589>.

**Standards, Standard Developing Organizations, OSAC**



### W13 Statistical Interpretation Software for Friction Ridge Skin Impressions: FRStat

*Anthony Koertner, MS\*, Forest Park, GA 30297; Henry J. Swofford, MSFS\*, Forest Park, GA 30297*

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**Learning Overview:** After attending this workshop, attendees will be familiar with a novel method for calculating the strength of fingerprint evidence using a statistical interpretation software, FRStat, developed by the United States government.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by providing participants with the knowledge, skills, and abilities to provide a statistical foundation to latent print examinations using FRStat software to ensure examinations are conducted and conclusions are articulated in a more scientifically defensible manner.

Over the years, the forensic science community has faced increasing amounts of criticism by scientific and legal commentators, challenging the validity and reliability of many forensic examination methods that rely on subjective interpretations by forensic practitioners. As noted in 2009 by the National Research Council (NRC) of the National Academies of Science (NAS) and more recently by the President's Council of Advisors on Science and Technology (PCAST), a main concern is the lack of any empirically demonstrable bases to substantiate conclusions from pattern evidence. This limits the ability for the judiciary to reasonably understand the reliability of the expert's testimony for the given case. Consistent with several academic commentators, both the NRC and PCAST strongly encouraged the forensic science community to develop tools to evaluate and report the strength of forensic evidence using validated statistical methods. While these concerns apply to nearly every pattern-evidence discipline, the forensic fingerprint discipline has received most of the attention because fingerprint analysis is one of the most widely used techniques in the criminal justice system. As a result, numerous methods and models have been proposed to provide a statistical estimate of the weight of fingerprint evidence. However, *none* have been widely accessible to the forensic community, thus prohibiting their ability to be further evaluated or implemented into routine casework. Consequently, forensic science laboratories throughout the United States have been unable to adequately address the concerns raised by the NRC and PCAST by demonstrating the reliability of fingerprint evidence for the case at hand.

Over the past few years, the Defense Forensic Science Center's (DFSC) United States Army Criminal Investigation Laboratory (USACIL) has taken incremental steps toward the development, validation, and implementation of a method, FRStat, which facilitates the evaluation and reporting of the statistical strength of fingerprint evidence. In March 2017, the USACIL implemented FRStat into routine casework and began reporting the statistical strength of fingerprint evidence within the military criminal justice system. Now, FRStat is the only method known to be in operational use within the United States that provides the capability of ensuring that the strength of fingerprint evidence is evaluated with an empirically grounded basis.

Through a combination of lectures, group discussions, and practical exercises, this presentation will provide an overview of rudimentary statistical concepts relevant to FRStat, discuss the development, validation, and implementation of FRStat, and provide instruction on how to appropriately interpret, report, and testify to the FRStat results.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the United States Department of Defense or United States Department of the Army.

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#### Fingerprints, Statistics, Probability



### W14 Classifying Unknown Human Mandibles Using the Statistical Procedures and Worldwide Databases Available in (hu)MANid

*Gregory E. Berg, PhD\**, DPAA Identification Laboratory, Joint Base Pearl Harbor-Hickam, HI 96853-5530; *Michael W. Kenyhercz, PhD\**, Department of Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI 96816

**Learning Overview:** After attending this presentation, attendees will better understand how to use (hu)MANid, a free, web-based Graphic User Interface (GUI) that allows the user to classify the sex and ancestry of an unknown mandible. Attendees will learn about the three primary statistical classification models available in GUI: linear discriminant analysis, mixture discriminant analysis, and random forest modeling. In-depth discussions detailing how to take appropriate measurements and score the morphology will be presented, culminating in hands-on practice using provided specimens and follow-on classification using the program.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the understanding of how metric and morphological data from the human mandible can be used for determining sex and ancestry for unknown specimens via the free, easy-to-use GUI, (hu)MANid.

This workshop is designed to improve the ability of the forensic anthropologist to conduct sex and ancestry assessments using the human mandible via a relatively newly released GUI, (hu)MANid. As is well known, most of the prominent categories of the biological profile (sex, age, and ancestry) can be addressed from the mandible, yet it has received little attention over the years. Indeed, many computer programs have focused on the cranium for sex and ancestry assessment, but none have effectively addressed the potential of the mandible for making these determinations. Further, few, if any, programs incorporate both metric and morphoscopic data into the same model. (hu)MANid fills this gap in the forensic anthropologist's tool kit.

(hu)MANid is an R-based GUI that allows the user to quickly and easily process mandibular metric and morphoscopic data. The GUI provides the user with the ability to classify a mandible into one of 26 different geographic or temporal reference populations, or into one of several composite global groups. Access to (hu)MANid is easy through the internet and is available freely at [www.anthropologyapps.com](http://www.anthropologyapps.com). It is not necessary to have R installed on the host computer to use this program.

Prior research has demonstrated that the mandible has a high accuracy rate for sex estimation and depending on the group(s) and statistical approach used, very high classification rates for ancestry estimation. Tests using (hu)MANid have shown the mandible to achieve sex and ancestry estimations on par with many currently available methods. Using random forest modeling, newly released in (hu)MANid, mandibles often classify to their respective groups with greater than an 85% accuracy rate (depending on the number of variables available for analysis and groups selected). Finally, the intra- and inter-observer error rates are very low for metric measurements and morphological scores of the mandible, indicating a high reliability between practitioners across cases.

As with any new tool, it has been found that there is a learning curve associated with it—partly from the additional statistical capabilities that this program has over the more standard and familiar forensic applications, as well as from some common misunderstandings of how to measure and score mandibles. With these issues in mind, this workshop will cover the following areas: how to appropriately measure and score a mandible; how to use the (hu)MANid (e.g., everything from how to enter a case to how to print out the results); a discussion on discriminant analysis (both linear and mixture) do's and don'ts, introducing the new capability of random forest modeling within the GUI; and suggestions on case workflow to effective results. The mistakes on measuring and scoring mandibles, result interpretations, statistical considerations, and the use of non-standard data collection possibilities will be highlighted.

The workshop is comprised of lecture and hands-on training that will cover everything from data collection to analysis and reporting of results by the attendees. This will provide the opportunity to (re)learn skill sets that may not be used often, practice those skills on a variety of mandibles, and practice using (hu)MANid for data analysis. Initial data collection and analysis will be conducted at the attendee level, then an interactive discussion with the entire audience will be held to generate additional questions and answers. Experienced practitioners will be available throughout the hands-on portion to answer questions, provide instant feedback, and discuss options.

#### Mandibles, Classification Programs, (hu)MANid



### W15 So You Want to Publish? A Basic Primer and Introduction to Forensic Publishing

*Rachel Hamilton Walton, EdD\**, Price, UT 84501; *Katherine M. Brown, PhD\**, Tarleton State University, Bryan, TX 77807; *Mark A. Listewnik, MA\**, Taylor and Francis, Boca Raton, FL 33487; *Jenny Cossham, MS\**, John Wiley & Sons Ltd, Chichester, West Sussex PO19 8SQ, UNITED KINGDOM; *Michael A. Peat, PhD\**, The Woodlands, TX 77381; *Alexander J. Smith, MA\**, Elsevier, Kidlington, Oxfordshire OX51GB, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will have a basic understanding of how to: (1) determine whether their proposed topic is suitable for publication; (2) identify and contact a publisher; and (3) work with the publisher through the publication process. Participants will attain a foundation of journal and book publishing. In addition, attendees will be able to identify the differences between publishing styles and formats and the differences in book, chapter, or journal requirements.

**Impact on the Forensic Science Community:** Publication of research and findings is essential for open review and discussion by those engaged in the forensic sciences. This presentation will impact the forensic science community by educating participants who desire to publish their forensic work in the solicitation, acquisition, and editing phases of the process for journal or textbook publication. This presentation will assist in furthering communication and enhancing awareness among scientific professionals and disciplines.

Having published a textbook or peer-reviewed journal article is a professional accomplishment that acknowledges your skill and expertise in a topical field. Publication not only establishes or furthers writing credentials, it may assist in professional promotion or other recognition of the work. Academia usually requires publication for granting tenure and promotion, and publication in the *AAFS Journal of Forensic Sciences* or other areas that further the forensic sciences may assist in meeting criteria for membership promotion within the Academy.

The forensic sciences are in a continuous state of discovery, evaluation, and re-evaluation of old and new forensic practices and techniques. As new knowledge is gained, it needs to be shared with the forensic science community. Publication in chapters, books, and peer reviewed journals is a primary means by which information is disseminated. It is most likely that forensic students and practitioners across the international spectrum have, at one time or another, thought about publishing in a peer-reviewed journal or a textbook. But lack of knowledge on how to proceed further has mystified and undoubtedly inhibited many qualified forensic-oriented personnel from pursuing this avenue. The questions so often asked are: “How do I go about being published?” “Where do I start?” “Where do I go?” “How can I get help?” and “Who would be interested in my topic?”

This introductory workshop will answer these questions. It offers the participant the opportunity to obtain valid, first-hand answers to these hurdles, and more. A panel comprised of representatives of forensic textbook and journal publishers will discuss questions proposed by a moderator. After each representative has had an opportunity to respond to each question, attendees will have the opportunity to further pose questions from the floor. Attendee participation is strongly encouraged. Panel members will present their perspectives on determining whether a prospective author has a marketable topic and how to locate an appropriate publisher. The panel will guide attendees, in detail, through the start-to-finish aspects of forensic publishing. This will include how to approach a publisher and suggested do’s and don’ts at this stage. Attendees will learn of the various types of editors involved in the publication process, from solicitation of the manuscript to those involved through the review and proof process, the final editing stages, and then publication.

As journal submissions are typically much different than those submitted for textbook format, the panel will offer further, specific guidance for consideration and writing for a peer-reviewed journal submission. The discussion will clarify the difference and importance between a peer-reviewed manuscript and one which is not subject to such. For both text and journal submissions, the attendee will further become aware of some of the legal requirements for publishing as well. The panel will address the importance of credits and source citations and documentation. Inclusions such as photographs or similar exhibits have separate permission requirements, and these will also be discussed.

Upon completion of this workshop, the attendee will have a better, more comprehensive understanding of what is involved in the publishing process. This presentation will de-mystify the publication procedure and attendees will be better able to assess and review their work and their thoughts about publication and will have the basic knowledge to make their dream publication a reality.

The presence of these representatives of forensic textbook and journal publishers is not an endorsement of their products or their firms by the American Academy of Forensic Sciences, and all statements and opinions are their own and do not reflect the views of the AAFS.

#### **Publishing, Forensics, Processes**



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### W16 Litigating Fingerprint Evidence: Ensuring Transparent and Sound Scientific Foundations

*Henry J. Swofford, MSFS\*, Forest Park, GA 30297; Anece Baxter-White, JD\*, Defense Forensic Science Center/USACIL, Forest Park, GA 30297; Brendan P. Max, JD\*, Cook County Public Defender, Chicago, IL 60602*

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**Learning Overview:** After attending this workshop, attendees will better understand the basic tenets of scientific validity, understand how statistical methods may be applied to non-DNA disciplines with specific emphasis on fingerprint evidence, and be better equipped to litigate cases involving pattern evidence domains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing legal and scientific practitioners to key issues concerning the examination, interpretation, and testimony of forensic pattern evidence domains and discuss a roadmap for litigating forensic evidence to ensure expert testimony is transparent, empirically substantiated, and scientifically valid. This workshop will include recommendations for laying the foundation for the introduction of pattern analysis evidence and discuss the challenges for its admission.

Fingerprint evidence has been admissible in legal proceedings for more than a century and practiced by nearly every forensic laboratory throughout the world. Once viewed as the gold standard of forensic evidence, the fingerprint discipline, in addition to nearly every other forensic discipline, is experiencing a great deal of turbulence as it navigates through the criticisms from the National Research Council (NRC), the President's Council of Advisors on Science and Technology (PCAST), and several other professional working groups and academic commentators. Although the forensic fingerprint discipline was determined to be foundationally valid by the PCAST in 2016, questions remain regarding the validity of the methods when applied to a specific case at hand. The primary concern is the legal field's inability to assess the reliability of fingerprint comparison results for a given case without validated statistical data concerning the strength of the findings, thus bringing into question the scientific validity of fingerprint evidence and threatening its admissibility in criminal courts. These concerns have stimulated a great deal of healthy debate within the forensic fingerprint discipline regarding how forensic science laboratories can move forward considering these criticisms and demonstrate the applied validity of their methods so they may continue to serve the criminal justice community.

Through a combination of lectures and group discussions, this workshop will provide a candid assessment of the current state of the forensic fingerprint discipline through the lenses of scientific validity, discuss existing gaps between the current state of the practice and the ideal future state of the science, and propose a way forward for the forensic fingerprint community to navigate toward a stronger scientific foundation. As a result, forensic science practitioners, laboratory leaders, forensic science policy makers, and criminal and civil litigators will have a much better understanding of the issues related to traditional practices of fingerprint examinations, become familiar with novel methods that can be leveraged by forensic science laboratories to promote more objective, transparent, and standardized practices, and become better positioned to litigate and introduce forensic evidence and advocate for appropriate improvements within their respective jurisdictions.

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#### Litigation, Fingerprint, Scientific





### **W17 A Think Tank on the Leading Edge of Forensic Science: Big Data, Head Trauma, Risk Evaluation, Drones, Video Analysis, and Disruptive Technology**

*Laura L. Liptai, PhD\*, BioMedical Forensics HQ CA/FL, Moraga, CA 94556; Zeno J. Geradts, PhD\*, Netherlands Forensic Institute, Den Haag, SH 2497 GB, NETHERLANDS; Curtis B. Coulter, JD\*, Law Offices of Curtis B. Coulter, PC, Reno, NV 89501; Victor W. Weedn, MD, JD\*, George Washington University, Washington, DC 20007; Anthony M. Hallett\*, Unmanned Response, Inc, Beaver, PA 15009; Stephanie Domitrovich, JD, PhD\*, Sixth Judicial District of PA, Erie, PA 16501; Mary E.S. Case, MD\*, St. Louis County Medical Examiner, St. Louis, MO 63134; Scott Blair, JD\*, Brain Injury Law of Seattle, Edmonds, WA 98020*

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**Learning Overview:** After attending this presentation, attendees will better understand the various new advances in forensic science that may have an impact on their work. The goal of this presentation is to reveal new developments that revolutionize forensic science and explain how these developments may impact the work of forensic scientists. Practical examples will be presented on big data, head trauma, risk evaluation, drones, video analysis and disruptive technology. This presentation will provide an overview of exciting new advances and open a forum for the discussion of issues that may arise regarding these new developments that will ultimately impact the forensic science community. A wide variety of new technology that will soon impact forensic science has been identified within the Think Tank Committee of the Forensic Sciences Foundation, Inc.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the impact of new developments on big data, head trauma, risk evaluation, drones, video analysis, and disruptive technology.

The benefits of real-time, onsite forensic investigations are multidisciplinary. New technology can increase the speed and efficacy of the criminal justice system. However, the benefits of this technology can only be realized if the quality of the data can always be guaranteed and the findings can be admitted by the court as proper forensic evidence. Efforts are being undertaken worldwide to innovatively develop integrated forensic platform solutions that will allow for the investigation of human biological traces, the chemical identification of illicit drugs, and the study of large amounts of digital evidence. The technological advancements that are revolutionizing forensic science could lead to a paradigm shift in which a new role of the forensic expert emerges as the developer and custodian of integrated forensic platforms. A means to revolutionize resolution in court cases will be presented by a legal innovator.

Another exciting topic is the use of drones in forensic science. You will see both the challenges and the opportunities that arise and how to further integrate drones into the justice system. First responders regularly use drones to gather data and assess situations. The data obtained could benefit those in the forensic, medical, or insurance profession. However, drones are also being utilized to conduct criminal matters. As a result, a protocol for forensic scientists to examine captured drones is being undertaken.

Attendees will also investigate head trauma by studying the traumatic unconscious and how that affects forensic pathology as well as biomedical engineering. There are numerous cutting-edge advancements in medical and biomedical forensics that can change the way one addresses head trauma analyses.

Video footage can have a revolutionary effect on forensic engineering analysis. How can that footage be properly used and improperly abused? Captivating examples will be presented.

Last, attendees will dive into how technology can be disruptive and the effect it has on the judicial system. A Pennsylvania judge will provide insight into how judges and court personnel will adapt since the current legislature will not be able to anticipate every outcome of the changing technology.

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### **Big Data, Head Trauma, Technology**



## Workshops – 2019

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### **W18 Forensic Support of Fire Investigation: I Was Told There Would Be No Math**

*John L. Allen, PE\*, ATF, Ammendale, MD 20705; Julia A. Dolan, MS\*, ATF, Ammendale, MD 20705; David T. Sheppard, PhD\*, ATF, Beltsville, MD 20705; Raymond Kuk, MS\*, Forensic Science Laboratory, Beltsville, MD 20705*

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**CANCELED**



### W19 Multidisciplinary Techniques to Assist With Missing and Unidentified Persons Investigations

*Noelle J. Umback, PhD\**, Office of the Chief Medical Examiner, New York, NY 10016; *Richard M. Thomas, PhD\**, FBI Laboratory, Quantico, VA 22135; *Glenn K. Bard\**, PATCtech, Plainfield, IN 46168; *Lori Bruski, BS\**, University of North Texas Health Science Center, Fort Worth, TX 76107; *Lawrence A. Dobrin, DMD\**, Office of the Chief Medical Examiner, Roselle Park, NJ 07204; *Jonathan Hayes, MD\**, Office of the Chief Medical Examiner, New York, NY 10001; *Bryan Johnson, MSFS\**, Federal Bureau of Investigation, Quantico, VA 22135; *Aden G. Naka, MSc\**, Office of the Chief Medical Examiner, New York, NY 10016; *Angela Soler, PhD\**, Office of the Chief Medical Examiner, New York, NY 10016; *Brandon C. Letts, PhD\**, FBI Laboratory, Quantico, VA 22135; *Patricia J. Aagaard, BS\**, FBI Laboratory - CODIS Unit, Quantico, VA 22135

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**Learning Overview:** The goals of this presentation are to provide an overview of the different techniques available to generate potential investigative leads in missing persons cases and to assist with the identification of unknown individuals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of forensic and investigative techniques utilized to provide support to unidentified and missing persons investigations.

Every year, thousands of people are reported missing to law enforcement agencies while the nation's medical examiners and coroners catalog hundreds of cases involving unidentified human remains. Historically, investigators have utilized forensic techniques as well as investigative methodologies to link an unidentified decedent to a reported missing person. Oftentimes, it is necessary to employ a combination of techniques to obtain adequate information to support an identification. Periodic updates regarding technological advances and new applications allow experienced investigators, as well as novices, with the opportunity to consider additional strategies to assist with the identification process.

Workshop speakers include practitioners from state and federal agencies with specialized knowledge and experience in various disciplines including anthropology, latent print examinations, data basing, odontology, DNA, pathology, and digital media exploitation. Presentations will highlight advances in technology and new initiatives in the disciplines, as well as a variety of biometric repositories.

Anthropologists will discuss how skeletal material can provide biological profile information to narrow the list of possible missing person matches to unidentified decedents, as well as how documentation of skeletal anomalies and antemortem trauma can assist identification comparisons. Additionally, skeletal remains can provide the template for forensic artists to create facial approximations for media releases to generate investigative leads. Odontologists will explain how dental examinations and dental records contribute to the identification process. Pathologists will discuss the use of tattoos and body modifications.

Many identifications are aided using national databases containing biometrics. The utilization of latent print and DNA comparisons have become routine practices in unidentified and missing persons investigations. A new initiative that focuses on searching unknown deceased fingerprint cards will be explored. Additionally, the Combined DNA Index System (CODIS), which compares the DNA profiles obtained from unidentified individuals, missing persons, and relatives of missing persons, will be discussed, as well as new DNA technologies that will have an impact on human remains investigations.

Additional database resources will also be discussed, including the National Missing and Unidentified Persons System (NamUs), the National Crime Information Center's (NCIC) missing and unidentified indexes, and the Violent Criminal Apprehension Program (ViCAP).

A member from an Identification Unit will describe the importance of outreach efforts, such as Missing Persons Day events, and liaison with stakeholders, such as the National Center for Missing and Exploited Children (NCMEC).

New advances in digital media exploitation will be presented, which will include a discussion on tracking human trafficking on the "Dark Web."

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### Missing Persons, Unidentified Decedents, Human Identification



### W20 The Forensic Pathology of Gunshot Wounds

*Jane W. Turner, PhD, MD\*, St. Louis, MO 63104-2013; Nadia A. Granger, MD\*, Monroe County Office of the Medical Examiner, Rochester, NY 14586; Juliette Scantlebury, MD\*, St. Louis City Medical Examiners' Office, St. Louis, MO 63103; Marianna Eserman, MD\*, New Orleans, LA 70125; MariaTeresa A. Tersigni-Tarrant, PhD\*, Saint Louis University School of Medicine, St. Louis, MO 63104; Diane C. Peterson, MD\*, Office of the Jackson City ME, Kansas City, MO 64108*

**Learning Overview:** After attending this presentation, attendees will be able to: (1) classify typical gunshot entrance and exit wounds; (2) differentiate atypical gunshot wounds; (3) use new advances in gunshot residue analysis; (4) explain the pathophysiology of gunshot injury; (5) interpret bullet tracks given different scenarios; (6) integrate forensic anthropology findings in bullet injuries and tracks; (7) review typical and atypical suicide gunshot wounds; and (8) describe autopsy findings in an oral presentation as if testifying.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the competence of forensic pathologists, forensic anthropologists, and other practitioners in examining, describing, correlating, and documenting gunshot injuries.

This is a course on gunshot injuries and related topics in the practice of forensic pathology. Having knowledge and experience with the evaluation of gunshot wounds and related injuries is paramount in the practice of forensic pathology. In examining individuals with gunshot wounds, the forensic pathologist must decide as to the cause and manner of death. Experience and knowledge in assessing these injuries is beneficial in many cases in making these determinations.

The forensic pathologists and forensic anthropologist who are presenting the course have extensive experience in examining, evaluating, interpreting, and documenting gunshot injuries. The instructors in this course combined have more than 50 years of experience, have examined several thousand individual gunshot injuries, and have testified under oath in hundreds of criminal proceedings. The purpose of this course is to impart the knowledge of these experienced practitioners to those who have only occasional cases involving gunshot injuries and to those who wish to refresh their training and knowledge of gunshot wounds. The objectives of the course for the learner include distinguishing the different types of entrance wounds and exit wounds; identifying graze and tangential wounds and other gunshot-related wounds; describing gunshot injuries, both typical and atypical; recognizing atypical gunshot wounds and artifacts; observing findings related to gunshot injuries, including microscopic findings; assessing old gunshot wounds and understanding the limits of interpreting entrance and exit wounds in some cases; applying forensic anthropology to the interpretation of trajectory and injury; correlating scene findings with autopsy findings; appraising new practices in the evaluation of gunshot residue; distinguishing the various firearm types, including those that are homemade; recognizing patterns of injury involving gunfire into a motor vehicle; determining the cause of death in cases of multiple injuries; evaluating gunshot wounds in cases involving law enforcement; interpreting radiographs; providing interpretations of trajectory given different hypothetical scenarios as in courtroom testimony; and evaluating typical and atypical suicide cases.

At the end of the course, participants will have the opportunity to participate in a practicum in which they review photographs of scenes and gunshot wounds and provide their own interpretations and written or oral reports. Participants will provide a report in which they describe the X-ray findings, location of injury, the type of injury based on the findings, the size and features of the injury, the track of the injury, the trajectory of the shot, the internal damage caused by and/or related to the injury, the presence or absence of a bullet, and the pathophysiologic effect of the injury.

#### Gunshot, Wounds, Pathology



### **W21 The National Transportation Safety Board (NTSB): Understanding and Preventing Impairment in Transportation**

*Robert L. Sumwalt III, MS\*, National Transportation Safety Board, Washington, DC 20594; Jana M. Price, PhD\*, National Transportation Safety Board, Washington, DC 20594; Nicholas Webster, MD\*, National Transportation Safety Board, Washington, DC 20594; Leah Walton, BA\*, National Transportation Safety Board, Washington, DC 20594; Philip M. Kemp, PhD\*, Bioaeronautical Research Lab, Oklahoma City, OK 73169-6901; Russell Lewis, PhD\*, Oklahoma City, OK 73167; Marilyn A. Huestis, PhD\*, Huestis & Smith Toxicology LLC, Severna Park, MD 21146*

**Learning Overview:** After attending this presentation, attendees will be able to (1) describe how the NTSB conducts its investigations of aviation, marine, rail, and highway events; (2) know the role of toxicology in the investigations; and (3) understand how this knowledge leads to recommendations to improve transportation safety and prevent impairment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the role of impairment in NTSB investigations and the importance of toxicology in understanding the contribution of drugs to impairment.

In this workshop, the NTSB's role in improving transportation safety, a historical overview, and current issues in transportation safety, as well as the goals for the agency and its future, will be discussed. The steps the NTSB investigators take both while on scene at an event and afterward to document the information necessary to understand potential impairment issues will be defined. With examples from recent and historical NTSB investigations, the methods to collect and document evidence, including operator and witness interviews, medical and pharmacy records, toxicological evidence, in-vehicle data, cell phone data, and physical evidence from the crash scene, will be provided. The criteria applied in the NTSB investigations to determine if operator impairment contributed to the cause of a crash will be explained. Three case studies will provide examples of drug impairment: a truck driver impaired by a prescription benzodiazepine and cannabis; a commercial balloon pilot impaired by a psychiatric condition and multiple prescription medications; and a pilot of a light civil aircraft impaired by the effects of prescription and over-the-counter sedating antihistamines and heart disease. The impact of safety recommendations on transportation will be explained. Three brief investigations produced safety recommendations regarding drug impairment and drug testing. The status of these recommendations, as well as the advocacy actions addressing driver and operator impairment are illustrated. The NTSB Most Wanted List items, such as impairment, which are eligible for additional advocacy support from the NTSB Board Members and staff, will be explained, and the resources to further promote these items and drive implementation of the NTSB safety recommendations provided. The toxicological road map for analyzing biological specimens collected in NTSB investigations will be outlined. The wide variety of biological specimens and analytes required, their advantages and disadvantages, especially in trauma cases, required instrumentation, and recent trends and potential future impairing drugs will be included.

Toxicological data from several challenging NTSB cases will be described to illustrate specimen testing procedures. Technical aspects of how forensic toxicology is used in the analysis of NTSB investigations to develop probable cause will be illustrated. Toxicological findings in specific cases and their contribution to probable cause will be portrayed. In an Amtrak® train rail crash involving a backhoe in Chester, PA, multiple drugs and medications were identified in several individuals. The role the drugs played in the event and the determination of probable cause are discussed. In the conclusion, the drugs did not contribute to the crash.

In the second case, the role of medications and the determination of probable cause of a light airplane crash in Abilene, TX, are described. This case involved coordinated testing between two laboratories and the conclusion was that the identified drugs directly contributed to pilot impairment and were part of the probable cause. Onsite biological specimen collection and testing now and in the future and how this might be helpful for NTSB investigations will be presented. The advantages and disadvantages of alternative matrices and their role in transportation safety and the difficulties in interpretation in these cases will be discussed.

#### **NTSB, Drug Impairment, Human Performance**



### W22 The Use of Dental Morphology in Forensic Anthropology

*Marin A. Pilloud, PhD\**, University of Nevada, Reno, Reno, NV 89557-0096; *G. Richard Scott, PhD\**, University of Nevada, Reno, Reno, NV 89557-0002; *Rebecca L. George, MA\**, University of Nevada, Reno, Reno, NV 89506; *Christine M. Pink, PhD*, Metropolitan State University of Denver, Denver, CO 80217-3362

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**Learning Overview:** After attending this presentation, attendees will be able to identify various morphological traits of the dentition that are relevant to studies of human variation and forensic anthropology. Attendees will also be able to apply this knowledge to the estimation of ancestry using a newly developed web-based application. Additionally, attendees will be introduced to a new database designed for the collection of dental data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with applied experience in the recordation of dental morphological variation and the application of these data in a statistical framework in which to estimate ancestry.

Dental morphology has been broadly used in biological anthropology to study human variation and explore research questions centered on migration, social structure, and macro- and micro-evolution. Despite this research, these data have not traditionally been used within forensic anthropology outside of the trait-list approach. However, recent research has shown that dental morphological data have much to offer the field of forensic anthropology.

This workshop will center on the use of dental morphological data and how it can be used as part of the biological profile. There are now methods to employ these data to estimate ancestry within a robust statistical framework utilizing a worldwide reference sample. As these traits have not been widely used in the discipline, training in the identification of dental morphological variants is lacking, and analysts have been hesitant to incorporate these data into their analyses. While observer error is certainly an issue in employing these traits, with proper training, dental morphological data can easily be included into forensic anthropological casework and research.

Presentations will begin with an explanation of the systems used to record dental variation and will outline the main traits used in describing human variation. Proper tooth identification will also be briefly addressed. Analytical methods will then be discussed to include the correct application of these data and statistical analyses. Attendees will be introduced to a custom database designed to record these data as well as to a statistical application to estimate ancestry. Following these presentations, attendees will be provided the opportunity to apply this knowledge in the analysis of skeletal material and casts. Participants will be guided through the process of dental trait scoring using the custom database presented, as well as using the web-based application to estimate ancestry.

Dental morphology represents another aspect of the phenotype, and therefore the genotype, that can aid in the identification of an unknown individual. Understanding the application of these data provides forensic anthropologists with one more method to employ in skeletal analysis, which may be particularly important in difficult or fragmentary cases.

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#### **Ancestry Estimation, rASUDAS, Dental Non-Metrics**



### **W23 How to Measure and Reduce Drug Background Levels in Forensic Laboratories and Their Implications on Safety and Data Integrity**

*Edward Sisco, PhD\**, NIST, Gaithersburg, MD 20899; *Marcela Najarro, MFS\**, National Institute of Standards and Technology, Gaithersburg, MD 20899; *Amber Burns, MS\**, Maryland State Police Forensic Sciences Division, Pikesville, MD 21208; *Kendra Broadwater, MS\**, National Institute for Occupational Safety and Health, Cincinnati, OH 45226; *Robert Kirkby, MS\**, Michigan State Police, Lansing, MI 48913; *Daniel E. Katz, MFS\**, Maryland State Police, Pikesville, MD 21208; *Theresa DeAngelo, MS\**, Maryland State Police Forensic Sciences Division, Pikesville, MD 21208; *Joaquin Cabrera, JD\**, States Attorney's Office of Worcester County, Snow Hill, MD 21863; *Susan M. Ballou, MS*, Gaithersburg, MD

**Learning Overview:** The goal of this workshop is for attendees to understand the significance and benefits of characterizing the composition and amount of drug background present in forensic laboratories. Attendees will learn: (1) about the recent work to establish drug background levels and how the data can be interpreted for the implications on health and safety and for data integrity purposes; (2) what processes contribute to a higher drug background and what controls and cleaning procedures can be implemented to reduce the potential for exposure; (3) what mechanisms laboratories are considering to improve analyst occupational health practices and maintain data integrity through changes to policies and procedures; (4) strategies for addressing drug background in court testimony; and (5) about analytical methods suitable to measure drug background levels and best practices required to collect and analyze the samples.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by providing drug analysts, quality managers, and laboratory directors with valuable information to make informed decisions on a range of topics, including identification of workflow processes that contribute to elevated drug backgrounds, cleaning protocols that reduce drug background, exposure prevention controls such as Personal Protective Equipment (PPE), and occupational health hazards from drug analysis.

Background monitoring is a common practice in many industries, such as pharmaceutical, food manufacturing, and healthcare, designed to address a wide range of challenges, from cross contamination to health and safety concerns to regulatory and data integrity. Although background monitoring has not typically been implemented in forensic laboratories, significant value can be drawn from knowing this data. For instance, the increased potency of drugs (i.e., opioids) has led to the need for more sensitive analytical instruments capable of detecting these drugs in trace amounts; therefore, characterizing the environmental background is critical. The basis for this workshop is a National Institute of Standards and Technology (NIST) -led, multi-agency collaboration focused on establishing drug background levels in forensic laboratories and interpreting the data for implications on health and safety and the data integrity of drug analysis. This workshop will provide foundational data on drug background levels measured in more than 20 forensic laboratories. This study quantified background levels using a targeted Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) drug panel. Samples were collected from a variety of surfaces in drug chemistry units, evidence-receiving units, and other units to establish the surface concentrations throughout each laboratory. These measurements identified workflow processes that most contribute to background, such as taking net weights of bulk powders. This presentation will inform attendees as to how laboratories are considering using this information to enhance their quality assurance programs.

Measuring background levels leads to the question: What do these levels mean for the health of analysts and for the data integrity of drug analysis? The National Institute for Occupational Safety and Health's (NIOSH) Health Hazard Evaluation (HHE) program has begun working with forensic laboratories to answer the first question and will be discussing the results of this work and providing broader suggestions regarding controls to improve analyst occupational health practices. To address the second question, the NIST is working with forensic laboratories to develop methods to quantify the limits of detection and reporting thresholds of their instruments such as Gas Chromatography/Mass Spectrometers (GC/MS). The goal here is to determine whether background levels present concerns for the data integrity of drug analysis. Additionally, efforts to quantify the efficacy of different cleaning agents, such as OxiClean™ and Dahlgren Decon, to reduce background will be discussed.

The final component of this workshop will provide participants with hands-on demonstrations and practice on how to properly sample surfaces within their laboratories to collect background. Following best sampling practices is crucial to the accuracy of measurements and their interpretation. In addition, attendees will be shown how Lateral Flow Immunoassays (LFIs) may be a robust and inexpensive way for laboratories to self-monitor and make nominal background measurements.

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#### **Drug Analysis, Background, Occupational Health**



### W24 Adult Skeletal Age Estimation: Transition Analysis Using the Entire Skeleton

*Stephen D. Ousley, PhD\**, Mercyhurst University, Erie, PA 16546; *George R. Milner, PhD\**, Pennsylvania State University, University Park, PA 16802; *Jesper L. Boldsen, PhD\**, ADBOU, Institute of Forensic Medicine, 5260 Odense S, DENMARK; *Svenja Weise, PhD\**, Institute of Forensic Medicine, Odense, DENMARK; *Sara M. Getz, PhD\**, Idaho State University, Pocatello, ID 83209-8005

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**Learning Overview:** After attending this presentation, participants will: (1) learn why attention must shift to many traits distributed throughout the skeleton to improve age estimation; (2) understand the analytical approach needed to use these age-informative traits effectively; and (3) acquire a basic comprehension of how to apply the procedure for generating age estimates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing an accurate and precise method of age estimation from the skeleton. Standard and frequently used procedures have long been known to yield inaccurate, biased (especially beyond 50 years), and imprecise age estimates. The new procedure, based on a wide range of skeletal features and refined analytical methods, yields far more accurate age estimates throughout all of adulthood. Attendees will learn how to apply the newly developed procedure, from scoring traits to generating age estimates.

The new version of the Transition Analysis procedure introduced here, based on ca. 1,700 individuals from four continents, yields estimates of age throughout the adult lifespan that are suitable for forensic use. The integrated approach features age-informative traits located throughout the skeleton, incorporates improvements in the analytical approach to make full use of those traits, and features a computer program to implement computationally intensive procedures.

This workshop begins with the logic and challenges of age estimation procedures, followed by a description of age-informative skeletal traits, the definitions of which were refined through examinations of multiple collections, often over a period of years. Most of the skeletal structures will be unfamiliar to forensic anthropologists who use standard methods focusing on pelvic joints, cranial sutures, and sternal rib ends. The skeletal traits are largely scored as either absent or present, which simplifies and speeds up scoring skeletons while reducing inter-observer error. Also discussed is the lengthy process of selecting traits that yield sufficient age-related information for inclusion in the procedure. Analytical procedures are then covered, including the importance of age-at-transition curves that yield age-specific probabilities of moving from one stage to the next (typically absent to present). Then, the use of the computer program for data entry and analysis are covered, including trait selection tailored to each skeleton. Results from the new program are covered, including the use of regionally specific and global (all population) reference samples.

This session ends with hands-on exercises in which participants score traits and estimate age from skeletons. This part of the workshop will provide opportunities to review and amplify points raised in previous lectures, and it will provide participants with a taste of what it is like to deal with real cases.

Although the analytical procedure is computationally complex, the overall goal has been to make it simple to use. Entering data in the program is straightforward, and the choice of which traits yield the most information in specific forensic cases are made automatically by the program. Thus, the program facilitates the best use of available data for human remains that are incomplete.

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#### Biological Profile, Skeletal Age Estimation, Transition Analysis





## Workshops — 2019

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### **W25 Low-Light Macro-Photography at the Crime Scene**

*Kevin J. Parmelee, PhD\**, Northeast Crime Scene Institute, Piscataway, NJ 08854; *Lauri Traub, JD\**, The Law Office of Lauri Traub, LLC, Saint Paul, MN 55101; *Barry Jansen\**, BMJ & Associates, Pittstown, NJ 08867

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**CANCELED**



### W26 Aspects of DNA Admissibility Hearings From the Prosecution, Defense, and Crime Lab Perspective

*Craig O. O'Connor, PhD\**, NYC Office of Chief Medical Examiner, New York, NY 10021; *Florence Hutner, JD\**, NYC Office of Chief Medical Examiner, New York, NY 10016; *Kristin Schelling, MS\**, Lansing, MI 48913; *Danielle Grootenboer, JD\**, Bergen County Prosecutor's Office, Hackensack, NJ 07601; *John D. Schmid, JD\**, Duluth, MN 55802

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**Learning Overview:** After attending this workshop, participants will better understand the general aspects of preparing for a DNA admissibility hearing from the lab, prosecution, and defense perspective. The panel discussion will include information and techniques that the various speakers use to prepare for and conduct hearings.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by introducing participants to the challenges faced in preparing for and conducting hearings and the overall impact of the admission or preclusion of various scientific techniques in a court of law.

Although admissibility hearings have long been a part of the judicial process, with the improvement of DNA testing techniques including enhancements in sensitivity that permit the accurate testing of lower amounts of DNA, the use of increasingly sophisticated statistical analysis software, and greater sophistication on the part of various stakeholders in the criminal justice system, the number of motions for admissibility hearings and actual hearings has increased. As with any admissibility challenge, the result of the hearing not only affects the case at hand but also has, in some circumstances, far-reaching impact on future techniques and cases.

The past 25 years have seen great strides in forensic DNA analysis. Improved evidence collection procedures and technology advancements have enabled scientists to provide previously unimaginable scientific evidence in criminal cases. Next generation platforms, test systems, and methodologies now provide scientists with the tools to reliably evaluate biological samples that consist of degraded, trace, touch, and/or contact DNA evidence. This enables scientists to address questions that were never thought of just a decade ago, including in court. The ability to give statistical weight to these different types of samples have greatly aided the judicial system but, like DNA testing in its infancy, have also led to admissibility questions. Educating the prosecution and defense bar has become an integral part of the lab analyst's duties. Additionally, articulating difficult molecular biology and statistical concepts to a layperson in a court of law has also become more challenging.

This panel of subject matter experts will present their thoughts and experiences as they prepare for and go through admissibility hearings. Topics covered will include contacting experts, gathering journal articles and court rulings, summarizing validation documents, as well as crafting questions and responses that will adequately convey the reasons why the technique is or is not scientifically sound and generally accepted in the relevant scientific community. This panel of experts will also discuss case studies, highlighting the challenges that were faced and how they overcame them.

Topics presented include: (1) general types of evidentiary admissibility hearings; (2) *Frye/Daubert* application to DNA evidence; (3) validation of techniques in a lab that will lead to their introduction in a court of law; and (4) preparation for a hearing.

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#### **Admissibility Hearings, *Frye/Daubert*, Courtroom Challenges**



### W27 Ethanol in Forensic Casework: Strategies for Analysis and Interpretation

*Robert Kronstrand, PhD\**, National Board of Forensic Medicine, Linköping, Östergötland SE 587 58, SWEDEN; *Nikolas P. Lemos, PhD\**, University of California-San Francisco, Palm Springs, CA 92262-6451; *Laura M. Labay, PhD\**, NMS Labs, Willow Grove, PA 19090; *Sherri L. Kacinko, PhD\**, Willow Grove, PA 19090

**Learning Overview:** The goals of this presentation are to provide the attendees with knowledge regarding ethanol absorption, distribution, and elimination in humans and to provide a scientific basis for the interpretation of postmortem ethanol findings, as well as for the estimation of Blood Alcohol Concentrations (BACs) from given doses and from analytical results in the living.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by: (1) providing current knowledge in the field of ethanol interpretation, thus enhancing the qualifications of forensic toxicologists practicing clinical and postmortem toxicology; and (2) increasing the competency of other disciplines, such as medical examiners and lawyers/attorneys.

The focus of this workshop is interpretation of ethanol (and some other volatiles) and its direct metabolites and markers in driving under the influence cases, violent crimes, and autopsy cases.

It is accepted that a toxicologist may report an extrapolated ethanol concentration in a blood sample collected from a living person. The context is usually traffic accidents or sexual assaults in which the blood sample is obtained several hours after the incident. A prerequisite is that all ethanol has been absorbed and that the person is in the elimination phase. A common approach for back extrapolation of ethanol concentrations is to use population-derived elimination rates. However, using population data to calculate an individual BAC provides only an estimation. Both underestimation and overestimation are equally important to recognize depending on the type of case. Therefore, the toxicologist must not only appreciate these shortcomings but also communicate them to the court.

The intra- and inter-individual variation in ethanol kinetics after single and repeated intake of different types of alcohol will be discussed using data from both controlled studies and case work.

A toxicologist may also be asked to estimate the dose of ethanol from body burden calculations or calculate the maximum BAC arising from a certain dose. Again, these calculations are subject to intra- and inter-individual variation in absorption and distribution caused by drinking behavior, gastric emptying, volume of distribution, and other factors that all should be considered when making such estimations. The scientific basis for estimations of dose and BACs will be discussed in this context.

Ethanol is produced postmortem when bacteria and fungi ferment substrates, primarily glucose, to produce energy. The glucose is metabolized to pyruvate, then converted to acetaldehyde. The acetaldehyde is then reduced to ethanol and transported out of the microbe. Ethanol is the main product of microbial fermentation. However, acetaldehyde, acetone, acetate, 1-butanol, and N-propanol are examples of other volatiles produced.

In the presentation on postmortem ethanol analysis and interpretation, presenters will discuss the formation of ethanol and other volatiles and discuss strategies for investigating this using different matrices and direct ethanol metabolites, such as ethylglucuronide and ethylsulphate and considering the formation of other compounds. Results from controlled studies as well as data from cases will form the basis for the strategies proposed.

#### Alcohol, Ethanol, Interpretation



### W28 Forensic Multimedia Authentication: Real-Life Challenges and Solutions

*Catalin Grigoras, PhD\*, Denver, CO 80202; Zeno J. Gerads, PhD\*, Netherlands Forensic Institute, Den Haag, SH 2497 GB, NETHERLANDS*

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**Learning Overview:** After attending this workshop, attendees will: (1) be familiar with the latest developments in forensic video and audio authentication; (2) understand criteria used for media authentication; (3) understand how to conduct analysis within a forensic framework; and (4) understand the current challenges and scientific solutions.

**Impact on the Forensic Science Community:** This workshop will impact the forensic science community by: (1) explaining the scientific approach in forensic media authentication; (2) demonstrating an authentication investigation framework; and (3) discussing tools used to combat multimedia forgery.

Digital multimedia authentication seeks to determine the validity of digital multimedia containers and contents by investigating their format, structure, time, frequency, and pixel and/or sample level features. This workshop will discuss the multimedia authentication process providing the user with methods of authenticating both video and audio. It will also demonstrate the incorporation of multiple tools and techniques into unified frameworks appropriate in forensic examinations where reducing examiner bias and error is crucial.

This workshop will cover video and audio authentication analyses. The goal is to provide an overall view of conducting comprehensive examinations that rely on the results of multiple analyses to inform an ultimate finding or opinion. First covered is a video authentication framework, focusing on camera verification/identification, and image and video attack detection. Photo Response Non-Uniformity (PRNU) are small artifacts of the sensor and can be used as a type of fingerprint for the sensor. For video and images, it can be determined with a high likelihood that a certain image or video has been made with a specific camera. PRNU can also be used for detecting deep fakes. Splicing, copy-move, and removal artifacts are also investigated in a complex video authentication process and will be discussed and exemplified with original and manipulated videos.

In the second section of this workshop, real-life audio challenges and solutions will be presented. The proposed audio authentication framework combines both container and content analysis to determine authenticity of the recording as well as the purported source. Audio container analysis will exploit characteristics of the multimedia file format and structure while content analysis will cover time and frequency domain techniques, including quantization level, power, direct current offset, butt splice, long-term averaged spectrum, long-term averaged sorted spectrum, compression level, and Modified Discrete Cosine Transform (MDCT) analysis.

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#### Multimedia, Forensic, Video and Audio

### A1 Quantification of Age-Related Pubic Symphyseal Morphological Changes Based on the Analysis of Clinical Multi-Detector Computed Tomographic (MDCT) Scans in Malaysian Males

*Salina Hisham, MSc\*, The University of Western Australia, Crawley, Western Australia 6009, AUSTRALIA; Nurliza Abdullah, MPath, Hospital Kuala Lumpur, Kuala Lumpur 50586, MALAYSIA; Mohamad Helmee Mohamad Noor, MS, Baltimore, MD 21223; Daniel Franklin, PhD, University of Western Australia, Crawley 6009, AUSTRALIA*

**Learning Overview:** After attending this presentation, attendees will be familiar with a succinct description of ongoing research designed to quantify age-related morphological changes that occur in the pubic symphysis based on the analysis of clinical MDCT scans (resolution range: 0.75-1.5mm; 87% @ 1.0mm). The study sample comprises 165 adult males drawn from a contemporary Malaysian population, with an age range of 15-83 years (mean 37.82; SD  $\pm$ 17.69).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a preliminary evaluation on the application of the Suchey-Brooks method to a Malaysian sample, thus highlighting the issue of population variation and the need to a develop population-specific formula for forensic practice.

There is a paucity of population-specific data for Malaysian individuals due to a lack of documented human skeletal collections. As an alternative, clinical MDCT scan databases are retrospectively evaluated as a source of “bone data” for forensic anthropological studies. The pubic symphysis is known to be one of the most reliable indicators of adult skeletal age, based on the premise that the symphyseal surface of the pubis undergoes metamorphoses as age progresses. Even though it was developed based on an isolated American sample, the Suchey-Brooks pubic symphyseal aging method has been applied globally, including in forensic practice in Malaysia. Therefore, the goal of this study is to examine the relationship between age and morphological change in the pubic symphyseal face, as visualized in CT images of a Malaysian sample, assessed following the six-phase scoring system established by Brooks and Suchey.

Pelvic CT scans exhibiting normal pathology obtained from the Department of Radiology, Hospital Sultanah Aminah Johor, were retrospectively evaluated. The anonymized scans (only age, sex, and ethnicity data are retained) were received in Digital Imaging and Communications in Medicine (DICOM) format, then reconstructed into 3D images using RadiAnt™ DICOM Viewer 4.6.5. Visualization of the pubic symphyseal face was performed using 3D volume rendering and the required morphology scored following the Suchey-Brooks method. Statistical analyses were performed using IBM® SPSS version 25.0. Transition analysis (Nphases2 program) was also utilized to calculate age ranges for each of the defined phases.

Intra-observer error was quantified based on three repeated assessments of 50 individuals representing all age groups, with all evaluations conducted within a one-month interval. Intra-observer agreement is excellent ( $\kappa = 0.832$ ). Spearman’s rho denotes a high correlation between age and phase ( $r=0.947$ ;  $P<0.01$ ). These results indicate higher accuracy rates for all phases (SD  $\pm$ 1.36-8.77 years) compared to the original work by Brooks and Suchey in 1990 (Standard Deviation (SD)  $\pm$ 2.1-12.2 years). Transition ages between phases 0 and 1, 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 6 were reported as 18.95, 23.77, 29.24, 43.35, and 60.83 years, respectively (SD  $\pm$ 4.135 years). These preliminary findings provide further empirical evidence of the importance of population-specific anthropological standards, and a larger study is accordingly warranted to facilitate the formulation of robust Malaysian standards for forensic age estimation.

#### Age Estimation, Pubic Symphysis, Malaysia

### A2 An Evaluation of the Inter-Observer Reliability of Aging Methods From the Pubic Symphysis

Tyler E. Dunn, MS\*, Creighton University School of Medicine, Omaha, NE 68178; Sarah C. Kindschuh, PhD, Defense POW/MIA Accounting Agency Laboratory, Offutt Air Force Base, NE 68113; Brittany S. Walter, PhD, Defense POW/MIA Accounting Agency Laboratory, Offutt Air Force Base, NE 68113

**Learning Overview:** The goal of this presentation is to assess the inter-observer reliability of pubic symphysis scores for age estimation, to evaluate a potential area of error in pubic symphyseal aging, and to quantify the comparative inter-observer variation between a component and phase-based methods and among analysts with different levels of experience.<sup>1</sup>

**Impact on the Forensic Science Community:** This study impacts the forensic science community by reviewing the replicability of morphological assessments used for age estimation from the pubic symphysis and by validating the inter-observer reliability of these methods for use in forensic casework.

Any method applied to forensic casework must be proven valid and repeatable based on the *Daubert* standard; as such, an assessment of inter-observer reliability is necessary to ensure that these methods have replicability acceptable for use in a forensic context. Pubic symphyseal morphology has a long history of forensic applications and has the ability to provide relatively narrow age estimate intervals.<sup>2,3</sup> McKern and Stewart devised a component-based approach for age estimation from the pubic symphysis in which a summed composite score is calculated based on three independently evaluated morphological areas of the symphyseal face: (1) the Dorsal demiface (D); (2) the Ventral demiface (V); and (3) the symphyseal Rim (R).<sup>2</sup> The Brooks and Suchey method of age estimation from the pubic symphysis is a phase-based system, which relies on the overall assessment of the symphyseal face into one of six developmental phases.<sup>3</sup> The purpose of this study is to evaluate inter-observer reliability between these methods and to assess inter-observer reliability among analysts with differing levels of anthropological experience for each method.

The study sample consisted of 167 os coxae from United States service members from the USS *Oklahoma* loss at Pearl Harbor on December 7, 1941, identified at the Defense POW/MIA Accounting Agency (DPAA) Laboratory. All individuals were male, and damaged or pathologically altered pubic bones were excluded from analysis. Eight analysts independently assessed each os coxa in the blind. The analysts were divided into three cohorts: inexperienced ( $n=5$ ), experienced ( $n=3$ ), and a combined cohort of inexperienced and experienced ( $n=8$ ). The inexperienced cohort was comprised of individuals with some level of postgraduate anthropological training and no casework experience; the experienced cohort was comprised of DPAA forensic anthropologists with advanced degrees in anthropology and, at a minimum, one year of casework experience. Each os coxa was scored following the McKern/Stewart and Brooks/Suchey methods, and each analyst had access to the original publication and plastic casts for both methods. Inter-observer agreement was determined using an Intraclass Correlation (ICC) analysis to assess inter-observer reliability (mixed model and 95% confidence) and evaluated using the Cicchetti scale. ICCs were calculated for each cohort for the McKern/Stewart method component and composite scores and for the Brooks/Suchey method phases.

The lowest ICCs were consistently observed in the inexperienced cohort, while the experienced cohort demonstrated the highest ICCs. The inexperienced cohort had ICCs of 0.70 and 0.76, and the experienced cohort had ICCs of 0.86 and 0.88 for the McKern/Stewart and Brooks/Suchey methods, respectively. When observations were divided into the independent components of the McKern/Stewart method, the inexperienced group had ICCs of D:0.60, V:0.63, R:0.64, and the experienced group had scores of D:0.79, V:0.87, and R:0.76. The McKern/Stewart method ICCs were lower than the Brooks/Suchey method ICCs for all analyst cohorts.

All scores demonstrate ICCs that indicate good to excellent agreement, an indication that both approaches are broadly consistent across observers and that observations when using these methods are replicable. The differences between the experienced and inexperienced groups indicate that analysts are more consistent when they have more osteological experience and regularly apply the method. The consistently higher ICCs for the Brooks/Suchey method may be a result of detailed descriptions of the changes in symphyseal morphology provided for this method, compared to relatively sparse descriptions provided for the McKern/Stewart method. Within the experienced group, the symphyseal rim component of the McKern/Stewart system had the lowest ICC relative to the other two components, which is possibly a result of poor documentation and guidance for scoring this component in the original publication. Overall, the inter-observer agreement for developmental scores of the pubic symphysis are good to excellent, and these methods are recommended for forensic applications, barring large deviations between the reference sample and individual being assessed.

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2. McKern T, Stewart T. *Skeletal age changes in young American males analysed from the standpoint of age identification*. Natick, MA: Quartermaster Research and Development Center, Environmental Protection Research Division, 1957.
3. Brooks, S., and J.M. Suchey. "Skeletal age determination based on the os pubis: A comparison of the Acsadi-Nemeskeri and SucheyBrooks methods." *Human Evolution*, vol. 5, no. 3, 1990, pp. 227-238.

#### Age Estimation, Inter-Observer Error, Pubic Symphysis



### A3 Assessing the Utility of the Suchey-Brooks Method on a Historical Sample

Sarah C. Kindschuh, PhD\*, Defense POW/MIA Accounting Agency Laboratory, Offutt Air Force Base, NE 68113; Brittany S. Walter, PhD, Defense POW/MIA Accounting Agency Laboratory, Offutt Air Force Base, NE 68113; Tyler E. Dunn, MS, Creighton University School of Medicine, Omaha, NE 68178

**Learning Overview:** The goal of this presentation is to compare the accuracy, inaccuracy, and bias observed between the McKern and Stewart and Suchey-Brooks methods of pubic symphyseal aging.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by directly comparing various methods developed on historical and modern samples to assess the utility of each method in a forensic context, as well as by comparing the accuracy between a component-based and a phase-based system of age estimation.

In forensic anthropology, the last two decades have seen an increased focus on the development of population-specific methods to account for variation inherent in modern human groups. Given that it is best practice to use aging methods developed on a contemporaneous sample, forensic anthropologists use methods developed on either modern or historical reference samples, depending on when the individual died. For example, Defense POW/MIA Accounting Agency Laboratory analysts typically employ the McKern and Stewart (MS) component scoring method for WWII or Korean War (i.e., historical) cases, and the Suchey-Brooks (SB) phase-based method for Vietnam War cases or more recent casework. However, because of taphonomic changes to bone, the MS method, which requires assessment of three areas of the pubic symphysis, may not be applicable if damage is present on at least one of the components. This presentation examines the applicability of the SB method on WWII era individuals to determine whether it can be used in place of the MS method, by comparing the accuracy, inaccuracy, and bias between the methods.

The study sample consists of 33 left and right os coxae of 22 identified WWII-era United States service members from the USS *Oklahoma* loss at Pearl Harbor on December 7, 1941. Individuals are males aged 19-43 years at death. Eight observers with varying levels of experience independently scored each pubic symphysis in the blind using both methods (observations  $n=160$ ). Each observer had access to publications and plastic casts for both methods. Accuracy was assessed using correct classification rates for several estimated age intervals of both methods: SB 1 Standard Deviation (SD) from the mean, SB using 95% prediction interval, MS 1SD and 2SD from the mean, and the MS observed range (the total range of ages in which a composite score was observed in the sample). Observer inaccuracy and bias using the mean age were calculated to quantify the amount and direction of error, respectively.

The estimated age interval using the SB 95% prediction interval was the most accurate (90% correct classification); this is unsurprising, given the large size of these intervals. The accuracy of MS observed range is comparable to SB using 1SD (82% and 83% correct classification, respectively). Correct classification of MS using 2SD is 79%, while MS using 1SD is 56%. However, the SB method has a substantially higher SD compared to the MS method. Overall, correct classification rates indicate that if the pubic symphysis is damaged, precluding the use of MS, analysts can use SB with a similar level of accuracy, though the estimated age interval will be large.

Patterns of observer bias are consistent with previous evaluations of forensic casework.<sup>3</sup> Bias for MS indicates slight under-aging (-1.1 years), while bias for SB indicates over-aging (4.3 years). Examination of bias for each MS component or composite score shows no obvious pattern. However, for the SB method, bias generally increases with each subsequent phase (Phase 1=-1.5 years, Phase 6=27.7 years), which is an artifact of the age intervals increasing size with each subsequent phase. Within this study, overall inaccuracy is slightly higher than previously reported at 4.0 years and 6.3 years for MS and SB, respectively.<sup>3</sup> When examining inaccuracy of each component score or phase, there is a general trend of increasing inaccuracy for both methods as the scores or phases progress, though this is more reflective of decreasing precision (i.e., larger age intervals at older ages) rather than an increasing inaccuracy of the scores or phases.

Results presented here demonstrate that despite temporal differences of the samples, these methods can be used with similar levels of accuracy. This suggests that if casework involving historical era individuals is missing one or more components required for the MS method, the SB phase method can be used if enough of the pubic symphyseal face can be assessed.

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#### Age Estimation, Pubic Symphysis, Biological Profile



### A4 Topographic Analyses and the Estimation of Age at Death From the Pubic Bone

*Andrew C. Seidel, MA\**, Arizona State University, Tempe, AZ 85287-2402; *Christopher Stojanowski, PhD*, Tempe, AZ 85281; *Laura C. Fulginiti, PhD*, Forensic Science Center, Phoenix, AZ 85007; *Kristen Hartnett-McCann, PhD*, Office of the Chief Medical Examiner, Farmington, CT 06032

**Learning Overview:** After attending this presentation, attendees will understand the potential of topographic analyses for the development of new, objective, and fully quantitative methods for the estimation of age at death based upon age-related changes to the surface complexity of the pubic bone.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the relationship between the surface complexity of multiple regions of the pubic bone and age at death. Results in this presentation indicate that topographic analyses may be used to develop age-estimation techniques that overcome the limitations of qualitative methods and improve upon the accuracy of current quantitative methods, especially for older decedents.

This research was conducted using the Hartnett-Fulginiti collection curated at the Forensic Science Center in Maricopa County, AZ. This collection comprises more than 600 specimens of pubic symphyses from decedents of known sex, age at death, and ancestry. A sample of pubic bones from 20 individuals (9 females, 11 males) with ages at death ranging between 18 and 84 was selected for analysis. Selection was based solely upon age at death and not upon the degree to which bones conformed to the morphological expectations associated with their known age.

All pubic bones in the sample were scanned using an Identica Blue 2015 laser scanner, and the resulting 3D meshes were partitioned into two regions of interest: the symphyseal face and a portion of the ventral surface. Remnant cartilage adhering to some bones resulted in poor scan quality and yielded final sample sizes of 16 left ventral surfaces (6 female, 10 male) and 18 right symphyseal faces (8 female, 10 male). Scans for each region were given the same orientation and all meshes were reduced to 15,000 faces for computational purposes. The surface complexity of each mesh was quantified using the metrics of Relief Index (RFI), Oriented Patch Count (OPC), and Dirichlet Normal Energy (DNE) as implemented in the molaR package developed for the R statistical environment. The values for each of these metrics were then evaluated for their association with known age at death.

While the combined-sex sample sizes used in this study are small, they are capable of recognizing correlations whose magnitudes exceed  $r=0.61$  (for the symphyseal face) and  $r=0.64$  (for the ventral surface) with a power of 0.8 and at a significance level of  $\alpha=0.05$ . Even with these limitations, age at death is significantly correlated with RFI and OPC on the symphyseal face (RFI:  $r=-0.67$ ,  $p$ -value=0.002; OPC:  $r=0.64$ ,  $p$ -value=0.004) and significantly correlated to RFI and DNE on the ventral surface (RFI:  $r=0.66$ ,  $p$ -value=0.005; DNE:  $r=0.69$ ,  $p$ -value=0.003). The rate and trajectory of changes in surface complexity differ between the ventral surface and the symphyseal face. This suggests that a method for age estimation that takes into account multiple regions of the pubic bone may yield increased accuracy in comparison to current techniques. Modelling the relationships between these metrics and age at death as non-linear would likely improve upon these results, but the strength of these correlations (especially given the small sample size) suggest that RFI, OPC, and DNE can be profitably used in the development of a novel, fully quantitative method for the estimation of age at death. Moreover, these preliminary results suggest that quantification of the surface complexity of the ventral surface of the pubic bone may yield suitable estimates of age at death when the more commonly used symphyseal face has been damaged or is otherwise unavailable.

#### Age Estimation, 3D Laser Scans, Topographic Analyses





## A5 An Estimation of American Black and White Ancestry From Measurements of the Vertebrae and Sacrum

Rhian Dunn, MS\*, Erie, PA 16503; Luis L. Cabo, MS, Mercyhurst University, Erie, PA 16546

**Learning Overview:** After attending this presentation, attendees will understand the applicability of Ünlütürk's method to an American sample for ancestry estimation.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility for ancestry estimation of the metric measurements of vertebral elements utilized in Ünlütürk's method.<sup>1</sup>

Ancestry estimation is a key element of the biological profile utilized to aid in victim identification from skeletal remains. While the midfacial region has been shown to be the most accurate for ancestry estimation, cranial elements are often missing in forensic contexts. Thus, it is necessary to consider and validate ancestry estimation methods based on the postcranial skeleton, in terms of *Daubert* compliance. Currently, metric methods for the postcranial skeleton are relatively scarce. The most common metric tool for metric ancestry assessments, FORDISC®, uses linear discriminant function analysis to classify the unknown individual. But, while it includes 13 reference groups for the cranium, it only offers two ancestral groups for the postcranium, highlighting the disparity between the attentions traditionally paid to the two body regions in this sense. Some additional studies have looked into characteristics such as the anterior femoral curvature, which, as measured, was shown not to correlate strongly with ancestry, or measurements of the pelvic girdle, but the infrequent explorations of postcranial elements as an alternative to cranial ancestry assessment have also lacked validation studies.<sup>2,3</sup> The vertebral column has been studied for sex estimation, positive identification, age estimation, and stature, but its potential utility for ancestry estimation has received less attention, with the exception of Ünlütürk's studies. Ünlütürk defined a series of measurements and used them to extract sex-specific discriminant functions to classify Black and White South African individuals from the Pretoria Skeletal Collection. Ünlütürk's functions covered different vertebral and sacral combinations, not requiring the full vertebral column, and rendered classification accuracies up to 98.5%, suggesting a high potential for its forensic utility. The primary purpose of this study was to validate the use of Ünlütürk's method on an American sample. The ultimate goal was determining whether the method could be useful in American forensic settings, with or without modification of Ünlütürk's original equations.

A total sample of 251 individuals (with balanced sex and ancestry) from the Hamann-Todd Collection at the Cleveland Museum of Natural History were measured according to Ünlütürk's descriptions. An early 20<sup>th</sup>-century collection was selected because social constructs at the time the collection was started (i.e., segregation) may mimic social constructs at play when the Pretoria Collection was started (i.e., the apartheid), both potentially resulting in artificially inflated group differences due to reduced admixture and subjective removal or labelling of mixed or unclear individuals. Four of Ünlütürk's original ten functions were tested first in their corresponding sex equations (i.e., male dimensions were entered only in male equations), then in the pooled-sex equations in order to determine the need for sex-specific functions. Goodness-of-fit tests were used to assess whether the obtained accuracies departed from the null, random classification models, as well as to test for between-sex and between-ancestry accuracy differences, as the measurements in the original study had not been tested for specific differences between groups, and so classification in that study may have rather been expressing other confounding factors, such as body size.

The obtained classification accuracies in the sex-specific equations were not as high as those reported in Ünlütürk's original study (61%-70% in the American sample compared to the 80%-95% accuracies reported by Ünlütürk). More interestingly, the utilization instead of sex-pooled equations did not reduce accuracy (63%-71%), indicating that sex-specific equations may not be necessary in this method. The results of the goodness-of-fit tests strongly supported the same hypothesis, as all detected differences were related to ancestry ( $p < 0.001$ ), rather than to sex or to the interaction of sex and ancestry. This indicates that the obtained classifications are based on shape differences across ancestries, rather than on differences in size or on sexual dimorphism. In conclusion, Ünlütürk's method seems to be a valid and useful tool to detect actual ancestry differences, although it likely requires further studies and reference sample expansion to adapt it for a more general use and increase its accuracy in populations other than South African.

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### Ancestry Estimation, Metric Method, Vertebral Column



## A6 Ancestry Estimation Using Vertebrae: Koreans Versus United States Whites

Yangseung Jeong, PhD\*, MTSU, Murfreesboro, TN 37129; Heli Maijanen, PhD, University of Oulu/Archaeology, University of Oulu 90014, FINLAND

**Learning Overview:** After attending this presentation, attendees will understand that vertebrae have a potential to be used for ancestry estimation, particularly when discerning between Korean and United States Whites.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring the potential usage of vertebrae as an ancestry indicator, which has seldom been studied previously. Ancestry estimation using vertebrae will enhance the likelihood of identification of unknown victims, particularly when their crania are missing or seriously damaged.

Ancestry is one of the key components of the biological profile which forensic anthropologists reconstruct to identify unknown skeletal remains. Extensive effort has been made to estimate ancestry primarily using the morphological and metric features of the crania and teeth. Some postcranial bones have also been studied for this purpose, but the vertebrae still remain as one of the least-favored bones in ancestry estimation. However, the fact that body proportions such as the Cormic index (i.e., ratio of the sitting height to the total height) differ between populations implies that different populations would have different vertebral heights.<sup>1</sup> In this regard, this study explores whether vertebrae can make a meaningful contribution to ancestry estimation, specifically when Korean and United States Whites are to be discerned from each other.

Vertebral heights from C2 to L5 were measured from 317 Korean skeletons (184 males and 134 females housed in eight institutions of South Korea) and 215 United States White skeletons (109 males and 106 females at the William Bass Donated Collection of the University of Tennessee), following the instruction of Raxter et al.<sup>2</sup> Then, the discriminant function analyses were conducted on C2–L5, C-column (summed heights of C2–C7), T-column (summed heights of T1–T12), L-column (summed heights of L1–L5), and V-column (summed heights of C2–L5), respectively. Males and females were analyzed separately.

Overall, individual vertebral heights and column heights exhibited decent-to-good Correct Classification Rates (CCR). When it came to individual vertebrae, CCRs range 62.1% (C3) ~ 86.5% (L5) and 50.6% (C7) ~ 81.4% (L5) for females and males, respectively. Except for T5, females exhibit higher CCRs than males in all vertebrae by 1.3% (T3) ~ 17.8% (T10). CCRs of L-column (88.4% and 81.0% for females and males, respectively) were higher than the other columns or the whole vertebral column for both sexes. T-column marked the lowest CCRs (73.6% for females and 59.0% for males) among vertebral columns.

Based on the decent CCRs associated with the vertebrae and vertebral columns as well as the convenience of vertebral height measurement, it is expected that vertebrae can be used as a useful indicator for ancestry estimation. When all lumbar vertebrae are present, their summed height will be particularly useful to discern Korean from United States Whites for both sexes. It was also noted that L5 alone can produce as high CCRs as the L-column.

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### Ancestry Estimation, Vertebrae, Korean

## A7 Metric Variability in the Femur and Patella: The Potential for Ancestry Assessment

Marica Baldoni, MA\*, University of Rome Tor Vergata, Rome 00133, ITALY; Rachel Joseph, MSc\*, Liverpool John Moores University, Liverpool, Merseyside L3 3AF, UNITED KINGDOM; Carole A.L. Davenport, PhD, Liverpool John Moores University, Liverpool, Merseyside L1 9DE, UNITED KINGDOM; Constantine Eliopoulos, PhD, Liverpool John Moores University, Liverpool L3 3AF, UNITED KINGDOM; Matteo Borrini, PhD, Liverpool John Moores University, Liverpool, AE L3 3AF, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will understand the potential of femur morphology for ancestry assessment between European and African individuals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new method for ancestry estimation that could increase the chance to sort candidates for identification, even in highly fragmented skeletal remains.

The estimation of ancestry is a key attribute—and often the most difficult—of biological identity to be determined by forensic anthropologists in the examination of unknown skeletal remains.<sup>1,2</sup> Numerous approaches have been proposed, most focusing on the variations in cranial morphology among different geographic groups.<sup>3-5</sup>

Currently, ancestry assessment based on morphoscopic traits of the skull relies on a subjective trait list and observer experience.<sup>6-9</sup> Moreover, the wide range of possible taphonomic alterations and preservation conditions encountered in forensic materials can affect the availability of some skeletal elements, increasing the challenge of the analysis.<sup>10</sup>

Several attempts to estimate ancestry from postcranial elements have also been proposed, even though they are less commonly applied in the forensic context.<sup>2</sup> These methods rely mainly on differences in the anterior diaphyseal curvature and intercondylar shelf angle of the femur.<sup>11-13</sup> In addition, the dimensions and geometry of the proximal femur have gained some attention as potential indicators of ancestry.<sup>14-16</sup>

This study analyzed 60 individuals, 26 African Americans (13 males and 13 females) and 34 Caucasian Americans (16 males and 18 females), from the Terry Collection housed at the Smithsonian Institute in Washington, DC. A total of 12 measurements were taken from each individual. These were on the distal end of the femur and on the patella in order to evaluate differences in the joint morphology between the two population groups.

Student's *t*-tests were run for each measurement to check if measurements from the left and right sides could be pooled together. African American males exhibit no significant difference between the left and right side; therefore, measurements were pooled together. African American females exhibit significant difference for two measurements of the femur, while Caucasian Americans exhibit significant difference for one measurement in male and female individuals.

Linear analyses were performed for the groups that could be pooled to determine the sets of data useful for binomial logistics regression for ancestry prediction. A total of nine measurements (six describing the femur for males and three describing the femur and patella for females) have been selected for regression prediction; these measurements demonstrate a percentage of correct prediction ranging from 56.4% to 70.9% in the male sample and from 67.2% to 74.5% in the female sample. All statistical analyses were performed by IBM® SPSS Statistics v.24.

The goal of this project is to identify the presence of metric variation in the human femur and patella between African and European population groups; the results obtained provide regression formula with encouraging predictive values, demonstrating the need of further analysis on a larger sample involving both skeletal collections and data from living individuals.

In addition, this research demonstrates the potential forensic applications of this new methodology for ancestry estimation even in commingled and highly fragmented unknown skeletal remains.

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### **Ancestry, Osteometry, Biological Identification**



## A8 An Examination of Pelvic Scarring as a Determinant of Sex

*Helen M. Brandt, MS\*, Binghamton University, Binghamton, NY 13902-6000; Stephen P. Nawrocki, PhD, University of Indianapolis, Indianapolis, IN 46227-3697; Krista E. Latham, PhD, University of Indianapolis, Indianapolis, IN 46227*

**Learning Overview:** The goals of this presentation are for attendees to: (1) develop a better understanding of the factors influencing pelvic bone scarring; and (2) recognize the value of peri-auricular scarring in determining the sex of the decedent.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a statistically rigorous study of pelvic scarring using a large modern skeletal sample, helping to resolve the contradictory findings of prior research.

The following null hypotheses are tested: the preauricular sulcus, interosseous groove, pubic tubercle, and retroauricular surface do not covary with (1) parity, (2) sex, (3) age, or (4) body size.

Previous studies have obtained contradictory results regarding the association between pelvic scars and childbirth; most employed weak experimental designs, making it difficult to separate the factors that influence the formation of scarring. The first installment of the current study (presented at the American Academy of Forensic Sciences annual meeting in 2018) examined the relationship between parity and dorsal pubic pitting and found that the sex of the decedent is the primary determinant of pitting, while parity has a significant but low-level influence. The second part of this study examines four other pelvic scars, none of which displayed any relationship to parity in the original study. This study builds on prior research by using a large modern sample with known parity status, combining traditional qualitative scoring with quantitative measurements and using powerful Analysis of Covariance (ANCOVA) -family tests to separate the different influences of parity, sex, age at death, and body size. Consequently, more effective predictive models can be generated for use in forensic anthropological investigations.

The same sample of skeletons used in the previous study was assessed for the presence and degree of pelvic scarring. A total of 530 identified, primarily Euro-American individuals was drawn from the Texas State University Donated Skeletal Collection, the Maxwell Museum Documented Skeletal Collection, and the William M. Bass Donated Skeletal Collection. Sex, age at death, and ancestry were recorded for all specimens, and all females have self-reported parity status. Coxa height was used as an indicator of overall body size. The presence and severity of the preauricular sulcus and the interosseous groove were scored and the width, depth, and length of preauricular pits were also measured with sliding calipers. The type of preauricular sulcus and interosseous groove present (“groove of pregnancy” or “groove of ligament”) was also recorded. The height of the pubic tubercle was measured using a contour gauge and sliding calipers, and retroauricular surface rugosity was scored ordinally. Binary logistic or ordinal regression was used to analyze the categorical non-metric pelvic traits, and ANCOVA was used to analyze the continuous metric pelvic traits.

Results indicate that the presence of the preauricular sulcus and interosseous groove are strongly determined by sex and can serve as effective sex indicators. Females are more than twice as likely to present a preauricular sulcus (85%) or an interosseous groove (65%) than males (39% and 20%, respectively). Additionally, older individuals are more likely to display a preauricular sulcus than younger individuals. The type of preauricular sulcus is also affected by sex and age: females and older individuals are more likely to present a “groove of pregnancy” than males and younger individuals. Preauricular sulcus measurements are also affected by sex and age, with all dimensions being larger in females and male sulci becoming wider with age. However, in all tests, sex is by far the primary determinant of preauricular sulcus presence, type, and size, with age having only a minor influence. The type of interosseous groove does not differ by sex, suggesting that the distinction for this feature is morphologically irrelevant and/or it is too variable to be practically useful.

The height of the pubic tubercle is only affected by body/pelvic size in both sexes and thus cannot be used to determine the sex of unidentified individuals. The retroauricular surface increases in rugosity with age and body size and is more rugose in females, but age is the most significant determinant.

In sum, these results illustrate that the peri-auricular pelvic scars are useful primarily for determining sex, with the effects of other variables being minor or non-existent. The pubic tubercle and retroauricular area reflect increasing body size or age; none of the features examined here are influenced by childbirth.

### Forensic Anthropology, Pelvic Scarring, Sexual Dimorphism



### A9 The Impact of Antimeric Leg Length Asymmetry on Adult Stature Estimation: A Validation Study

Megan E. Ingvoldstad, PhD\*, DPAA Laboratory, Offutt Air Force Base, NE 68113; Brittany S. Walter, PhD, Defense POW/MIA Accounting Agency Laboratory, Offutt Air Force Base, NE 68113

**Learning Overview:** After attending this presentation, attendees will understand that antimeric leg length asymmetry is commonly encountered in forensic anthropological casework. Guidance is provided regarding how to estimate stature when asymmetry is present to avoid inaccurate stature estimates and erroneous exclusion of a decedent as a match to skeletal remains.

**Impact on the Forensic Science Community:** This presentation impacts the forensic science community by: (1) encouraging bilateral measurement-taking whenever possible to identify asymmetry; (2) cautioning against combining leg bone lengths from different sides to avoid compounding proportional antimeric asymmetry; (3) recommending the use of measurements from the right side; and (4) proposing selection of a 99% Prediction Interval (PI) when total leg length asymmetry equals 5.0mm. This practice can prevent inaccurate stature estimates and the erroneous exclusion of decedents from their skeletal remains.

Review of Defense POW/MIA Accounting Agency (DPAA) Laboratory identifications revealed that stature estimations are occasionally inaccurate due to the occurrence of antimeric total leg length asymmetry. Preliminary research presented at the American Academy of Forensic Sciences in 2018 specifically found: (1) antimeric total leg length asymmetries, up to 16mm, occurred in 68/78 (87%) cases, indicating asymmetry is common; (2) significant differences (5.0mm average) exist between antimeric total leg lengths; (3) the longer or shorter leg does not consistently produce a more accurate stature estimate; (4) when asymmetry is present, right leg measurements are consistently more accurate; (5) the left leg demonstrates reduced accuracy with elevated asymmetry; and (6) when a disparity between legs of  $\geq 5.0$ mm is encountered, increasing the PI from 95% to 99% ensures living stature will be captured (100% correct classification).

To validate these findings, stature data were collected from an independent sample of 20 adult individuals identified at the DPAA Laboratory in 2018. This sample includes males of European, African, Asian, and indeterminate ancestries, with ages at death between 18 and 43 years (mean=24.5 years, SD=3.7 years). Each individual had atraumatic and complete femora and fibulae present for analysis. Stature was estimated for everyone using FORDISC<sup>®</sup> 3 and the appropriate Trotter MSTATS male database. Maximum lengths of the left femur and fibula, and then the right femur and fibula, were used to calculate 95% stature PIs. These PIs were checked against the individual's antemortem stature (obtained by healthcare professionals during military medical evaluations) for accuracy. Stature estimates that did not include the identified individual's antemortem stature were run again using 99% PIs and assessed to see if the identified individual's antemortem stature was captured.

**Results:** (1) Total leg length asymmetries, up to 11mm, were observed in 90% of cases; (2) there is a statistically significant difference (mean=5.0mm) between antimeric leg lengths ( $p < 0.00$ ;  $t=6.5$ ); (3) use of the longer or shorter leg does not consistently produce more accurate stature estimates; (4) when asymmetry is present, the right leg is consistently more accurate in predicting living stature; (5) the left leg demonstrates reduced accuracy with elevated asymmetry; and (6) when a disparity between left and right legs of  $\geq 5.0$ mm is encountered, increasing the PI from 95% to 99% ensures the individual's living stature is captured (100%).

Overall, these results reinforce the preliminary findings that asymmetry is common between left and right total leg lengths, and that these asymmetries can be large enough to affect stature estimates and, therefore, identifications.

#### Biological Profile, Stature, Antimeric Asymmetry



## **A10 An Estimation of Stature Using Cranial Dimensions**

*Mubarak A. Bidmos, PhD\*, Doha 2713, QATAR; Abduljelil Adebisin, MSc, Sefako Makgatho Health Sciences University, Pretoria, SOUTH AFRICA*

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**THIS ABSTRACT WAS NOT PRESENTED.**



# Anthropology – 2019

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## A11 A Comparative Analysis of Stature Estimation Methods for Application in Forensic Anthropology

Susie C. Athey\*, Topeka, KS 66611; Mackenzie Walls, Forensic Anthropology Program, Topeka, KS 66621; Alexandra R. Klaes, PhD, Washburn University, Topeka, KS 66621; Heather M. Garvin, PhD, Des Moines University, Des Moines, IA 50312-4198

**Learning Overview:** After attending this presentation, attendees will better understand the accuracy of various stature estimation methods and their implications on the biological profile for identifying unknown individuals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comprehensive analysis of stature estimation methods and will identify the most accurate method for estimating stature for use in forensic contexts.

Forensic anthropologists use either anatomical or mathematical methods to estimate stature in unknown individuals. Anatomical methods use all bones contributing to stature and have been considered the most accurate approach. The popular mathematical methods use correlations of long bones to estimate overall stature and are not reliant on complete skeletal remains and are less time consuming. The ease of the computer program FORDISC® has likely contributed to the increased use of mathematical methods over anatomical methods, even with complete remains.

The first goal of this research is to validate each method type (anatomical vs. mathematical) and to determine if anatomical methods are in fact more accurate. The second goal of this research is to compare the accuracy of available methods and to determine if the methods derived from modern samples are more accurate for use with modern forensic cases. Skeletal measurements were collected for 72 White males and females of known stature from the William M. Bass Donated collection. A total of 41 measurements were collected from 36 bones by one expert observer, a practicing forensic anthropologist, using sliding calipers and an osteometric board to take the measurements of the individual. The left side was measured for each individual unless the bone was damaged or exhibited a pathology, in which case, the right side was substituted. The collected measurements were added together for the anatomical methods ( $n=2$ ), and the appropriate soft tissue correction factor was applied or entered into the sex and bone specific regression equations to estimate overall stature using the mathematical methods ( $n=4$ ). Age-correction factors ( $n=2$ ) were also applied and tested for accuracy. Lastly, estimated stature, with and without age correction factors, was compared to the known stature to determine the overall accuracy of each method.

Stature was more accurately predicted for females using both method types, despite having sex-specific equations in the mathematical methods, and male stature was typically underestimated in most methods. Overall, the revised methods for both the anatomical and mathematical methods provided more accurate estimates than the older methods, likely due to secular changes in body proportions. Raxter et al.'s revision of the Fully method provided a stature estimate closer to known height in 80.9% of individuals.<sup>1,2</sup> Newer mathematical methods (Ousley, Wilson et al.) produced higher accuracy rates per individually tested bones (76.4%-93.0%) than the older mathematical methods (Trotter and Gleser) with accuracy rates of only 50.7%-63.4%.<sup>3-6</sup> FORDISC® had a total accuracy rate of 91.7%, but with wide prediction interval ranges (average of 7.8 inches when using known sex and ancestry; 95%). Overall, the age correction factors did not improve accuracy and generally resulted in lower accuracy than the uncorrected estimates.

The results of this research suggest that the Wilson et al. method should be applied in forensic casework as it has the highest total accuracy, even outperforming anatomical methods, and small prediction interval ranges.<sup>4</sup> Generally, anatomical methods tended to underestimate reported stature, which may suggest that self-reported stature was likely overestimated, as first reported by Willey and Falsetti.<sup>7</sup> Overall, the tibia equations provided the best estimates of stature (93%) using Wilson et al.'s equations, followed by the humerus at 91.5%.<sup>4</sup> Wilson et al.'s tibia equation provided the most accurate estimates of actual stature overall (93.0%) and suggests that when possible, the tibia equation from Wilson et al. should be utilized for forensic casework over other stature methods.<sup>4</sup>

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### Stature Estimation, FORDISC®, Wilson Method



## A12 Tall Tales: Various Regression Stature Methods Applied to the Huntington Collection

Ashley Espinoza\*, Mercyhurst University, Erie, PA; David R. Hunt, PhD, Smithsonian Institution, Washington, DC 20013-7012; Gene Hunt, PhD, Smithsonian Institution, Washington, DC 20013-7012

**Learning Overview:** After attending this presentation, attendees will be aware of secular change in human populations and how these changes affect stature estimations, and, in this study, for European populations in which the individuals were born prior to the 1850s. This presentation will also demonstrate that the femur provides a more accurate stature estimation utilizing the four stature regression methods employed in this study.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community and the bioanthropological community by alerting them to be cognizant of the effects of secular change on the accuracy of stature estimations when applying these methods to populations that span more than a century.

Various regression formula stature methods have been created to estimate living stature for a specific population, by sex and/or ancestry. Those commonly used stature regression methods are Trotter and Gleser, Ousley, Wilson et al., and Albanese et al.<sup>1,3-5</sup> These methods measured individuals from anatomical skeletal collections coming from different time periods to develop their regression formulas. Trotter and Gleser used the Terry Collection consisting of individuals who were born in 1850 to 1900; Ousley had a mixture of individuals born pre-1944 and post-1944; Wilson et al. used individuals born post-1944; and Albanese et al. used individuals born from 1839 to 1914.<sup>1,3-5</sup> These methods, despite their common use, each have a level of error if applied to a population not used for producing their regression formulas. Even if all collections are of European ancestry, the variation due to secular change between these population groups will cause some error. This study tested the accuracy of Trotter and Gleser, Ousley, Wilson et al., and Albanese et al. regression stature methods by applying them to the femur and humerus of individuals from the Huntington Collection.<sup>1,3-5</sup> The Huntington Collection is composed of European immigrants born between 1827 and 1881 (pre-1900), thus being essentially a generation prior to the Terry Collection and earlier than the birth dates of the other collections used.<sup>2</sup>

The sample consisted of 49 individuals of known height: 27 males and 22 females. The sample size was somewhat restricted by the individuals that had recorded stature and both the humerus and femur from the same individual. The maximum length measurement of the humerus and femur were applied to each method for the White male and White female category. In all four methods, the femur had a lower average difference than the humerus. This reaffirms that the femur is the more effective element to use for regression stature estimation. This is, of course, due to its more direct contribution to height. The Albanese et al. femur regression formula provided the most accurate result with an average difference of 0.94 cm from the actual stature.<sup>1</sup> This is undoubtedly due to Albanese's sample collection time period being more similar to the Huntington collection's time period. The next accurate femur regression formula was Trotter and Gleser's (1.24cm), which used the Terry Collection.<sup>4</sup> Ousley and Wilson et al. had the highest average difference, with a difference of (1.81cm) and (FSTAT 2.28/ASTAT 2.43), respectively.<sup>3,5</sup>

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5. Wilson R.J., Herrmann N.P., Jantz L.M. Evaluation of Stature Estimation From the Database for Forensic Anthropology. *J. Forensic Sci.* 2010, 55 (3), 684-689.

### Stature Methods, Huntington Collection, Secular Change



### A13 Blast and Aircraft Crash Trauma: A Selection of World War II (WWII) Cases From the Defense POW/MIA Accounting Agency (DPAA) Laboratory

*Andrea Palmiotto, PhD\**, Indiana University of Pennsylvania, Indiana, PA 15701; *Kyle A. McCormick, PhD*, Defense POW/MIA Accounting Command, Joint Base Pearl Harbor-Hickam, HI 96853; *Traci L. Van Deest, PhD*, Defense POW/MIA Accounting Agency, Offutt Air Force Base, NE 68113; *Laurel E. Freas, PhD*, Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI 96853

**Learning Overview:** After attending this presentation, attendees will understand differential traumatic patterning in skeletal remains associated with historic wartime aircraft- and blast-related events.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by deconstructing analytical criteria associated with historic aircraft and blast events and providing guidelines to differentiate both types of trauma. These criteria will help anthropologists and pathologists working in mass disaster areas and possibly human rights work around the world.

Trauma assessment is a complicated endeavor that provides insight into the cause of death, peri-mortem interval, and life history of the individual. Because of their work with other forensic experts and in medicolegal situations, forensic anthropologists must demonstrate competency and accuracy in trauma analyses. As other researchers have noted, methods for trauma analyses require validation to ensure quality and scientific rigor of work.

Blast- and aircraft-related incidents are influenced by various extrinsic and intrinsic forces and represent extreme forms of bodily trauma that may result in similar patterns of injury. However, limited research has been completed regarding differentiating between these types of trauma, and no well-defined criteria exist for analysis.

Bodily trauma from propeller-driven aircraft crashes is characterized by extensive blunt-force trauma from deceleration. Anthropological literature categorizes four types of blast trauma, which result from different mechanisms and are associated with different fracture patterns. Bodily trauma from a blast event may result from the blast wave (primary), associated projectiles (secondary), acceleration/deceleration impacts (tertiary), or flash burns (quaternary). The potential injuries are characterized by a mixture of blunt-force and/or projectile trauma, resulting from exposure to explosive ordnance, such as grenades, landmines, mortars, and bombs. Furthermore, a set of remains may display complicated patterns that preclude identification of a single category of blast trauma.

Recently resolved WWII cases with established causes of death relating to blast ( $n=11$ ) or aircraft ( $n=24$ ) events were assessed. The reports were written by various anthropological analysts who performed their analysis in the blind. The causes of death were determined by a medical examiner based on the available historical and anthropological data. The blast cases are from open-environment ground losses, while the aircraft cases involve propeller-driven aircraft.

Among these cases, differences existed in the amount and overall condition of recovered remains, which may affect analytical potential. The majority of aircraft (50%) and blast (90%) cases reported at least 25% element recovery; complete biological profiles were possible for some aircraft (29%) and most blast (90%) cases. Widespread trauma was found predominantly in aircraft cases (66%) and less frequently in blast cases (27%), in which trauma tends to be more localized. Aircraft cases often displayed spiral (33%) and butterfly (20%) fractures, but none were observed in the blast cases. Multiple or indeterminate directionality was evident in all aircraft (100%) and a number of blast (45%) cases, but unidirectionality was observed only in blast cases (55%). Only blunt trauma was observed in the majority of aircraft cases (66%) and a smaller percentage of blast cases (36%), while other blast cases (45%) displayed some evidence of projectile trauma. Indeterminate trauma was observed in some aircraft (29%) and blast (9%) cases.

The results of this study suggest that physical trauma resulting from historic aircraft and blast cases can be differentiated based on the type and pattern of trauma present. Additionally, it may be possible to identify specific blast trauma categories within a set of remains; based on the analysis, blast trauma may be characterized as projectile (secondary) or non-specific.

Although numerous traumatic features may aid interpretations, they should not be used as definitive criteria. These case studies are intended to provide a comparative framework for differential diagnosis and possible points for future research. Similar fracture patterns may result from other causes, and not all criteria may be evident in all cases due to the inherent complexities of traumatic events and the recovery process, which affects the condition of remains present for analysis.

The views herein are those of the authors and do not represent those of the Defense POW/MIA Accounting Agency, Department of Defense, or United States government.

#### Trauma, Aircraft Deceleration, Blast Injuries



### A14 The Prevalence of Peri-Mortem Trauma Among Casualties of Armed Conflict

*Andrea Palmiotto, PhD, Indiana University of Pennsylvania, Indiana, PA 15701; Carrie B. LeGarde, MA\*, Defense POW/MIA Accounting Agency, Offutt Air Force Base, NE 68113*

**Learning Overview:** After attending this presentation, attendees will be aware of the prevalence of peri-mortem trauma among identified casualties lost during World War II (WWII), the Korean War, and the Vietnam War.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by testing expectations of and quantifying incidences of skeletal trauma for individuals associated with past United States armed conflicts. Although this analysis reflects one agency's casework, it provides insights on the expected prevalence of peri-mortem trauma observed in recovered skeletal remains in military, mass disaster, or human rights contexts.

These United States wartime casualties were lost under a variety of circumstances, including ground, air, and Prisoner of War (POW) losses. Evidence of peri-mortem trauma is expected, particularly in battle (i.e., ground) and air losses due to their engagement in armed conflict. Fewer cases of peri-mortem trauma are expected in POW losses since these deaths are more often attributed to malnutrition and illness.

A sample of 270 individuals identified since 2014 were examined for the presence of peri-mortem trauma and condition of skeletal remains. Peri-mortem trauma was observed in 128 (47%) cases. These cases were broken down by conflict, type of loss (ground, air, or POW), and proportion of the skeleton recovered.

The Vietnam War was represented by 20 cases, of which 16 were air and 4 were ground losses. These cases were recovered either in the field or through unilateral turnovers from foreign governments. All cases contained incomplete skeletons, with 90% of these cases missing most major elements and represented by less than a quarter of the skeleton. Peri-mortem trauma was observed in less than half of the cases (8/20): 44% of air and 25% of ground losses.

The Korean War was represented by 140 cases, of which 17 were air, 57 were ground, and 66 were POW losses. These cases were recovered predominantly through unilateral turnover, followed by disinterment of unidentified individuals from national cemeteries, and field recovery. Most cases were incomplete, and 44% of the cases were represented by less than a quarter of the skeleton. Peri-mortem trauma was observed in less than half of the cases (41/140): 47% of air, 51% of ground, and 6% of POW losses.

WWII was represented by 110 cases, of which 49 were air, 56 were ground, and 5 were POW losses. These cases were recovered predominantly by field recovery, followed by disinterment and unilateral turnover. About 34% of cases were represented by less than a quarter of the skeleton. Peri-mortem trauma was observed in most cases (79/110): 76% of air, 75% of ground, and none of the POW losses.

No peri-mortem trauma was observed in 142 (53%) cases; however, more than half (53%, 75/142) of these cases consisted of mostly incomplete skeletons. When broken down by loss type, 83% of air (25/30), 44% of ground (20/45), and 45% of POW (30/67) losses were mostly incomplete and lack peri-mortem trauma. In these cases, the absence of observed trauma may be due to the paucity of the remains, rather than indicating that no peri-mortem injury occurred.

As expected, air and ground losses displayed peri-mortem trauma more frequently than POW losses. Peri-mortem trauma was evenly represented between air and ground losses for both Korea and WWII cases, but it was observed more often among WWII cases than Korea or Vietnam cases. This is likely due to the completeness of the recovered remains. Remains were more often recovered and buried soon after the incident during WWII, giving some protection from taphonomic processes, unlike Korean and Vietnam War casualties. Although the Vietnam War is more recent, and one would expect that taphonomic processes may be less severe, these cases are the most incomplete. This could be due to factors such as soil pH of recovery location or loss type (WWII propeller-driven planes vs. Vietnam War jet air losses).

Identifying peri-mortem trauma is crucial for understanding the context of loss for missing servicemembers. These results indicate that despite wartime circumstances, not all skeletal remains exhibit evidence of peri-mortem bodily trauma; the absence of peri-mortem trauma cannot be used to determine loss circumstances. The absence of trauma may reflect recovery context or other taphonomic conditions, rather than the absence of skeletal injury.

#### Forensic Anthropology, Trauma, Taphonomy



### A15 Patterns of Intentional Dismemberment in Florida Medicolegal Death Investigations

Katie M. Rubin, MS\*, University of Florida, CAPHIL, Gainesville, FL 32610; Janet E. Finlayson, MA, University of Florida, Gainesville, FL 32610

**Learning Overview:** After attending this presentation, attendees will be familiar with the dismemberment modes, affected body regions, and implement types commonly observed at the C.A. Pound Human Identification Laboratory (CAPHIL).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enhancing the comparative data available for dismemberment cases.

Comprehensive data has been presented previously by Adams and Rainwater for New York City, a major metropolitan area; the data presented herein reflect non-comprehensive data for a single state.<sup>1</sup>

This study reviewed all CAPHIL case files accessioned between July 2003 and June 2018 ( $N=959$ ). The contents of each file were assessed for indications of intentional human dismemberment. During the 15-year period reviewed, the CAPHIL evaluated 31 cases—representing 28 individuals— involving intentional human dismemberment. These cases derived from 17 counties across the state, ranging from the southern peninsula to the Panhandle. There is an even split between male ( $N=14$ ) and female ( $N=14$ ) decedents in the sample. Ages at death range from neonatal to 76 years, with the greatest number of individuals falling into the middle-aged adult age bracket (30-49 years;  $N=14$ ). For the 24 individuals for whom a racial category is known, 22 are White and 2 are Black; this does not account for Hispanic ethnicity. This sex and race breakdown is relatively reflective of decedent demographics at the CAPHIL, as well as of the demographic breakdown of Florida.

All dismemberment methodology data derive from indications of dismemberment recorded on the skeletal elements submitted to the CAPHIL. Full sets of remains were not always available at time of analysis, due either to recovery circumstances or to selective submission of affected body portions by the medical examiner. Following Rainwater, the CAPHIL dismemberment cases were categorized by dismemberment mode: (1) anatomical disarticulation around joint articulations; (2) transection of bone via sawing; and/or (3) transection of bone via hacking.<sup>2</sup> Transection via sawing was the most common mode of dismemberment for the individuals assessed at the CAPHIL; 14 individuals fall into this category exclusively. Five individuals were dismembered solely by anatomical articulation, while only two individuals were dismembered solely by a hacking mechanism; six individuals were dismembered via a combination of modes, and the mode for one individual (the neonate) could not be determined. For all three cases involving sawing in conjunction with an anatomical mode of dismemberment, there was a partial (incomplete) transection through the spinal column.

Overall, the most commonly affected body regions were the neck ( $N=18$ ), left shoulder/upper arm ( $N=14$ ), left hip/upper thigh ( $N=12$ ), torso ( $N=10$ ), and right shoulder/upper arm ( $N=10$ ). The relatively high frequency of dismemberments through the torso seen in the CAPHIL sample is in contrast with the very low frequency of such dismemberments reported by Adams and Rainwater in their analysis of dismemberment cases in New York City.<sup>1</sup>

The most common implement was a saw used in isolation; multiple such cases display thermal alteration at transected margins, likely due to friction-generated heat produced by the action of the saw. A saw used in conjunction with a knife was the second most common means of dismemberment. Reciprocating saws were the most commonly used saw type; only one individual was dismembered using a manually powered saw. Two individuals exhibited tool marks from at least two different saws; both cases involved the use of rotary saws. All anatomical disarticulations used only a knife. Hacking implements ranged from heavy implements intended for hacking to improvised hacking implements, such as claw hammers and shovels.

Understanding dismemberment patterns from past cases is important for the advancement of future medicolegal investigations. Knowledge of common dismemberment practices may improve the total percentage of an individual recovered by informing upon what body portions may be anticipated given case circumstances. Trends in tool use may also aid law enforcement in preliminary searches for causative implements. Further, this information may better prepare forensic anthropologists to respond to unusual investigative requests; for example, the CAPHIL has been asked to compare dismemberment styles across multiple sets of remains to help law enforcement pursue possible connections between decedents in seemingly distinct cases.

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2. Rainwater C.W. 2015. Three Modes of Dismemberment: Disarticulation Around The Joints, Transection of Bone Via Chopping, and Transection of Bone Via Sawing. In *Skeletal Trauma Analysis: Case Studies in Context*. NV Passalacqua and CW Rainwater, eds, 222-245. Oxford, UK: Wiley Blackwell.

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#### Sharp Force, Decapitation, Dismemberment Mode



### A16 Skeletal Asymmetry of the World War II (WWII) Battle of Tarawa Skeletal Assemblage: The Impact on the Resolution of Commingling

Caryn E. Tegmeyer, PhD\*, Canton, MI 48187; Jesse Roberto Goliath, PhD, Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI 96853; Rebecca J. Wilson-Taylor, PhD\*, Joint Base Pearl Harbor-Hickam, HI 96853

**Learning Overview:** After attending this presentation, attendees will better understand the degree and prevalence of directional asymmetry within a commingled WWII battlefield population.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the need to understand patterns of asymmetry to improve segregation and association of remains in a commingled assemblage.

Skeletal asymmetry has been identified by many researchers within human populations and is identified as a lack of symmetry between two paired elements. Directional asymmetry occurs when this difference is apparent on one side of the body. Previous researchers have found that there is a right-sided bias in the directional asymmetry of the upper limb bones, and to a much lesser extent, a left-sided bias in the lower limb bones.<sup>1-3</sup>

Understanding asymmetry in commingled assemblages is important for the re-assessment of prior analyses and to facilitate the association of related skeletal elements for forensic identification purposes. Visual pair matching of skeletal elements is commonly employed by anthropologists to associate antimeres. Hence, any asymmetry could impact the ability of forensic practitioners to correctly and accurately match these paired elements. Minimal differences affect the overall size, shape, and morphology, thus biasing visual assessment. During the preliminary analysis of the Battle of Tarawa assemblage, a WWII battlefield population recovered from the Tarawa Atoll, Republic of Kiribati, asymmetries were noted between skeletal elements. This pilot study examined the magnitude that visual and metric asymmetries have on this unique population.

The maximum long bone lengths in both upper and lower skeletal elements (humerus, radius, ulna, femur, tibia, and fibula), as well as the maximum length of the clavicle were examined to determine the effects of directional asymmetry. Skeletal measurements, including the maximum length of these bones, were recorded following standard procedure at the Defense POW/MIA Accounting Agency. These records were consulted and if left and right measurements for any of these bones differed by 3mm or more, they were reported in a separate table. Following the formula provided by Auerbach and Ruff, a calculation of directional asymmetry was computed for each bone:  $\%DA = (\text{right-left}) / (\text{average of left and right}) \times 100$ , where a negative value suggests a left-side asymmetry and a positive value suggests a right-side asymmetry.<sup>2</sup> Values for each bone category were then averaged to obtain the percentage of directional asymmetry.

These data follow the pattern seen by previous studies of a right-side bias in the upper limb (with the exception of the humerus) and a left-side bias in the lower limb (with the exception of the fibula). The clavicle also displayed a left-side bias within this skeletal population. The lack of left-sided bias in the fibula can be explained by the fact that it is not a weight-bearing bone and therefore is less sensitive to the pressures of differential loading and remodeling. However, the absence of right-sided bias in the humerus is confounding. Based on previous data, and the presence of a right-sided bias in the radius and ulna, a left-sided bias in the humerus is an unexpected result and may be a result of selective occupational stress or overuse.

Following asymmetry determination, paired *t*-tests were conducted on each bone group to determine significance of direction asymmetry within the samples. In each case, the difference was found to be non-significant ( $p > 0.05$ ). This suggests that while asymmetry is present and affecting the skeletal measurements of some cases, the differences were not statistically significant across bone groups.

This study supports the need for both visual and statistical assessment when pair-matching elements. Even though some elements were deemed visually asymmetric, these observations did not correspond to overall differences between paired elements statistically. This is especially useful in dealing with the Battle of Tarawa assemblage, because it comprises a relatively homogenous population demographically, which limits the utility of some of the statistical pair-matching methods available.

The views of these authors do not necessarily reflect those of the Department of Defense or the United States government.

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1. Latimer, H.B. and Lowrance, E.W. (1965). Bilateral Asymmetry in Weight and in Length of Human Bones. *The Anatomical Record*. 152(2), 217-224. Auerbach, B.M. and Ruff, C.B. (2006). Limb Bone Bilateral Asymmetry: Variability and Commonality Among Modern Humans. *Journal of Human Evolution*. 50(2), 203-218.
2. Kujanová, M., Bigoni, L., Velemínská, J., and Velemínský, P. (2008). Limb Bones Asymmetry and Stress in Medieval and Recent Populations of Central Europe. *International Journal of Osteoarchaeology*. 18(5), 476-491.

#### Skeletal Asymmetry, Pair Matching, Commingling



### A17 Reassociating Commingled Human Crania With First Cervical Vertebrae: An Osteometric Approach

Vasiliki Louka, MSc, University of Athens, Athens, Attiki, GREECE; Ioanna Anastopoulou, BSc, University of Athens, School of Medicine, Athens 11527, GREECE; Konstantinos Moraitis, PhD\*, University of Athens, School of Medicine, Athens 11527, GREECE

**Learning Overview:** After attending this presentation, attendees will understand the importance of using osteometric methods in sorting commingled human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a statistically valid method of sorting skeletal remains from commingled assemblages, which can be supplementary to the traditional non-metric methods.

The sorting of human remains is crucial in situations where commingled remains are encountered, such as in mass disasters.<sup>1</sup> The cranium and first cervical vertebra are often found in commingled contexts. As the cranium is an anatomical region used for sex and age estimation, it is important to be attributed to an individual. The goal of this study is to provide a solid statistical method for reassociating commingled human crania with atlases using measurements taken from both skeletal elements.

For this purpose, the maximum bicondylar breadth, the maximum internal length and width of the foramen magnum, and the maximum length of the occipital condyles along their long axis were measured from 159 crania of the Athens Collection.<sup>2</sup> In addition, the maximum length and width of the vertebral foramen, the maximum length of the superior facets, and the maximum distance between the lateral edges of the superior facets were also taken.<sup>3</sup> The individuals included in this skeletal collection are of known sex, age, occupation, and cause of death. All specimens examined lived during the second half of the 20<sup>th</sup> century in Athens, Greece. The age of this sample ranged between 18 and 99 years.

Simple linear regression analysis produced a number of equations for reassociating the cranium with the atlas of the same individual using the aforementioned measurements. A total of five equations were appointed as the best statistical models for predicting measurements of one skeletal element using measurements of another. The chosen measurements presented a significantly strong correlation, with Pearson's correlation coefficient ( $r$ ) ranging from 0.73 to 0.88 ( $p < 0.05$ ). The coefficient of determination ( $r^2$ ) of the five models had a range of 0.56-0.77. The Standard Error of the Estimate (SEE) was between 1.26 and 2.15.

In conclusion, it is strongly believed that the regression models of this study are considered capable of matching the cranium and the atlas in a commingled assemblage. Further research on the application of the above method is recommended.

#### Reference(s):

1. Ubelaker D.H. Methodology in Commingling Analysis: A Historical Overview. In: Adams B.J., Byrd J.E., editors. *Recovery, Analysis and Identification of Commingled Human Remains*. Totowa, NJ: Humana Press; 2008; 1–6.
2. Holland T.D. Sex Determination of Fragmentary Crania by Analysis of the Cranial Base. *American Journal of Physical Anthropology*. 1986; 70(2):203–208.
3. Marino E.A. Sex Estimation Using the First Cervical Vertebra. *American Journal of Physical Anthropology*. 1995; 97(2):127–133.

#### Forensic Anthropology, Commingled Remains, Osteometric Sorting



### A18 Reassessing Determining Season at Death Using Dental Cementum Increment Analysis (DCIA) in Young Adults

Vicki Wedel, PhD\*, Western University of Health Sciences, Pomona, CA 91766; Timothy P. Gocha, PhD, Clark County Office of Coroner/Medical Examiner, Las Vegas, NV 89106

**Learning Overview:** After attending this presentation, attendees will be familiar with the method used to determine season at death using DCIA, presented with a reassessment of seminal (2007) data in comparison to data from two current studies. Attendees will be able to appreciate the methodological, histological, and microscopy skills necessary to apply this method.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing updated accuracy rates, as well as recommendations for the technological setup and analysis of dental cementum increment for season-of-death determination. This will inform future histological research, resulting in higher quality forensic research and practice.

In 2007, Wedel published a study in which dental cementum increments, or annulations, were examined in 112 teeth extracted from consenting oral surgery patients from Santa Cruz, CA.<sup>1</sup> Wedel reported that the season in which the tooth had been extracted (a proxy for date of death) could be determined to be between April-September (spring/summer) or October-March (fall/winter), with 99% accuracy.<sup>1</sup> The method used had been adapted from zooarchaeology and involved embedding the teeth, sectioning them on a low-speed saw, grinding and polishing the sections to 100 microns in thickness, examining them under transmitted, polarized light, and determining whether the outermost band was optically bright (spring/summer) or opaque (fall/winter).

Since 2007, others, including Meckel and Wescott, have attempted to replicate Wedel's results and been unable to achieve the 99% accuracy rate.<sup>2</sup> Meckel and Wescott report a 60% first-time and an 18% second-read accuracy rate from their analysis of teeth taken from 24 individuals aged greater than 50 years.<sup>2</sup> However, Meckel and Wescott did achieve accurate assessments of death in teeth from two individuals aged less than 22-years.<sup>2</sup> Meckel and Wescott urge caution in applying the method to teeth from individuals greater than 50 years of age but were optimistic that the method could be used in young adults.<sup>2</sup>

To look for corroborating or contrasting evidence of their results, this study re-examined teeth collected from the 18-25-year-old individuals in Wedel's original study. It is hypothesized that the outermost optically active annulation of extrinsic cementum can be distinguished from the outer edge of the tooth, and that the original seasons noted by Wedel will be distinguishable in this study as well.<sup>1</sup>

Forty teeth were available for this study, from which accurate assessments of season at death were made in 31 cases, for an accuracy rate of 78%. Two teeth were deemed indeterminate because of abnormal tooth histology, and this study was incorrect in its assessments of season in seven instances, five of which were teeth from transitional periods: late March through early April and late September through early October. This study, authored by two experienced hard-tissue histologists, agreed on all but 3 of the 38 teeth for which season was assessed, yielding an inter-observer agreement rate of 92%.

This presentation proposes to detail the fine-tunings of working with optically active tissue at the curved or obliquely oriented tooth edge using the polarized light and the fine focus mechanisms at 100x magnification. Included in the discussion will be the optimal tooth locations to observe the outermost band of optically active acellular extrinsic fiber cementum, in addition to limitations of applying this highly technical method to human teeth. Reasons for discrepancies between Wedel's and these results will be discussed, as will be the differences between this sample and Meckel and Wescott's sample.<sup>1,2</sup>

#### Reference(s):

1. Wedel, V.L. 2007. Determination of Season at Death Using Dental Cementum Increment Analysis. *J Forensic Sci.* 52(6): 1334-1337.
2. Meckel, L.A., Wescott, D.J. 2017. The Utility of Dental Cementum Increment Analysis for Estimating Season-of-Death in Naturally Decomposed Skeletons. Poster presented at the 87<sup>th</sup> Annual Meeting of the American Association of Physical Anthropologists-Austin, TX.

#### Dental Cementum Increment Analysis, Season at Death, Dental Histology





### A19 The Application of Textile Biodeterioration Rates to Postmortem Interval (PMI) Estimation

*Randi Marie Depp, BS\*, Mercyhurst University, Erie, PA 16546; Kellie Jones, BA\*, Las Vegas, NV 89130; Jennifer L. Webb, BS\*, Mercyhurst University, Erie, PA 16546; Jessica T. Novak, BA\*, Erie, PA 16504*

**Learning Overview:** The goals of this presentation are to provide a literature review of all studies regarding rates of clothing deterioration as well as a test of the concept using forensic cases from Mercyhurst University.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving data collection for textiles on a forensic scene and potentially improving PMI estimates.

Most attempts at estimating PMI rely on the physical condition of the human remains; however, the rate of decomposition is altered by many contributing factors, such as weather and season of the year. This greatly complicates PMI estimates. Given that the clothing an individual is wearing at the time of deposition is subject to the same elements as the remains, it is hypothesized that clothing will also exhibit a progressive rate of decomposition that could improve PMI estimates. In theory, textiles of the same composition should degrade along a predictable timeline, thus providing a way to estimate PMI. To date, very little has been produced in the way of textile research within the forensic sciences. Most studies that reference clothing do so only anecdotally, with a majority of the published research focusing on how the presence or absence of clothing affects the decomposition rate of remains.

The goal of this study is to provide a comprehensive literature review of all studies that discuss rates of clothing deterioration. As the studies are scattered across multiple journals and multiple fields, it is necessary that all relevant literature be synthesized in one place. This study also tests the concept on 19 forensic cases from 2006 to 2018 from Mercyhurst University to determine whether a significant correlation exists between the known or estimated PMI and the state of clothing deterioration. This test is twofold: first, it will apply textile degradation as an estimate of PMI to actual forensic cases, and, second, it will create reliable protocols and best practice guidelines for the documentation and assessment of clothing at forensic scenes.

As inconsistent scoring methods and a lack of well-defined procedures were the leading issues in most studies reviewed in the literature, well-defined protocols and a centralized source of comparative data will improve future endeavors. Mercyhurst cases were selected based on scene type and availability of textile information via photographs. The textiles were assessed based on amount of deterioration observed from photographs taken on scene or in the laboratory. Each type of material present was scored ordinally from 1-4, with 1 being 100% remaining, 2 being 99%-50% remaining, 3 being 49%-1% remaining, and 4 being 0% remaining (where the presence of a tag or zipper indicated that specific textiles had been present at one time). The data was then subjected to statistical analyses to determine if there was a correlation between the known or estimated PMI and the rate of biodeterioration.

Spearman's Rank Correlations were calculated utilizing Past 3.20 software to test the relationships between the minimum, maximum, and average PMI and biodeterioration rate. Power analysis was conducted with G\*Power software to determine the target sample size. Four separate Spearman's Rank Correlation tests were conducted with  $\alpha$  set to 0.05. Unfortunately, no conclusions can be drawn about the relationship between textile biodeterioration rates and PMI with the current limited sample size. However, a potential pattern was observed for the blended textiles category. The  $p$ -value for this material was 0.47143 and  $r$  was 0.34718. While this result is also not significant, it suggests that given a larger sample size, a relationship may exist. Many cases were available for analyses that fit this study's parameters; however, cases or textiles excluded from this analysis were lacking in at least one of two areas: (1) documentation of textile composition (typically found on a tag); and (2) clear overall photographs of the textiles spread out to determine the amount of deterioration. It is anticipated that textile biodeterioration rates and PMI will be significantly related given more scorable data and improved documentation and assessment protocols.

#### **Biodeterioration, Textiles, Postmortem Interval**



### A20 Best Practices for Macerating Cartilage

Alexis M. LaGoy\*, Baldwinsville, NY 13027; Tessa Somogyi, MA\*, Binghamton University, Binghamton, NY 13902; Elizabeth A. Evangelou, MA\*, Binghamton University, Binghamton, NY 13905; Elizabeth A. DiGangi, PhD\*, Department of Anthropology, Binghamton, NY 13902

**Learning Overview:** After attending this presentation, attendees will understand the need to adopt minimally destructive and easily available methods for removing extraneous soft tissue from thyroid cartilage, which is frequently damaged by tools or harsh methods during the macerating process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing useful, realistic, and minimally destructive methods for the removal of soft tissue from cartilage, identified via the use of store-bought raw pig ears as proxies for human tissue.

The thyroid cartilage is the uppermost cartilage in the human larynx, serving the purpose of supporting and protecting the vocal cords, as well as providing an attachment site for laryngeal muscles and the hyoid bone via the thyrohyoid membrane. In forensic contexts, visible abnormalities on the thyroid cartilage may be indicative of trauma, especially in forensic cases involving asphyxiation. The thyroid cartilage may bear ligature marks, broken superior horns, or even scratches. These can aid in determining the cause and/or manner of death.<sup>1</sup> Similarly, the position of ligature marks on the thyroid cartilage may distinguish between a suicide by hanging or homicidal strangulation. The thyroid cartilage must be cleaned and free of surrounding soft tissue before these marks can be clearly observed.

While much has been written in the literature about processing bones, little information exists about a proper way to process the thyroid cartilage. While cool water maceration will remove extraneous soft tissue from the cartilage, this method often takes weeks to months. Manual removal of soft tissue is a much faster process; however, it requires the use of tools, which may result in inadvertent cut marks.

The study described here tested several methods to process cartilage from 40 pig ear portions. Pig ears were chosen as a proxy for human thyroid cartilage because they are approximately the same thickness as human thyroid cartilage and are readily available at supermarkets. Unlike thyroid cartilage, which is a firm hyaline cartilage, pig ears are composed of a slightly more malleable elastic cartilage. However, both hyaline and elastic cartilage are composed of type II collagen and possess perichondrium, making them similar enough to use for the purpose of this experiment. The pig ears were quartered and cut to mimic the average dimensions of a thyroid cartilage. Ears were first subjected to sharp force trauma with a scalpel, producing cuts 3cm in length without completely penetrating the cartilage, before processing to determine if the processing method damaged the cartilage or cut mark in any observable fashion.

Four processing methods were utilized. These methods included: (1) dermestid beetles; (2) cold water maceration; (3) heat maceration; and (4) chemical maceration with 300mL solutions of (a) laundry detergent, (b) sodium borate, (c) bleach, (d) multi-purpose cleaner, (e) meat tenderizer, (f) papain, and (g) hydrogen peroxide. Four portions of pig ears were used to test each processing method.

A 19-point scoring system adapted from Steadman and colleagues was used to test the efficacy of the maceration methods.<sup>2</sup> The scores are based on odor (0-3), soft tissue texture (0-3), ease of soft tissue removal (0-5), cartilage quality (0-5), and duration (0-3). For each item in the scoring system, a higher number indicates best-case scenario (i.e., a method scoring a 3 for odor will have generated little to no odor). Methods receiving a higher point score are deemed better because they do well in multiple categories.

With a final score of 18/19, a solution of 1tsp of sodium borate to 300mL water at 150°F for two hours was found to be the best method for removing the surrounding tissue from pig ear cartilage, as it was quick, cost effective, and required no processing tools for soft tissue removal. In contrast, hydrogen peroxide earned a score of 4/19 as this method compromised the integrity of the pig ear cartilage.

This research provides a foundation for future testing of best practice methods for removing surrounding tissue from human thyroid cartilage.

#### Reference(s):

1. Mariam Arif. Ligature Mark on the Neck; How Elucidative? *The Professional Medical Journal*. 22, no. 6 (2015): 798-803.
2. Dawnie Wolfe Steadman, Lisa L. DiAntonio, Jeremy J. Wilson, Kevin E. Sheridan, and Steven P. Tammariello, The Effects of Chemical and Heat Maceration Techniques on the Recovery of Nuclear and Mitochondrial DNA from Bone. *Journal of Forensic Science*. 51, no.1 (2006): 11-17.

#### Forensic Anthropology, Maceration Methods, Cartilage



### **A21 Integrating Forensic Anthropology and Cold Case Databases: A Look at Resolved Unidentified and Missing Persons Cases in Louisiana**

*Emily F. Wieggers, MA\*, LSU FACES Laboratory, Baton Rouge, LA 70803; Teresa V. Wilson, PhD, Louisiana State University, Baton Rouge, LA 70803; Ginesse A. Listi, PhD, LSU Geography & Anthropology, Baton Rouge, LA 70803; Maria T. Allaire, MA, LSU FACES Laboratory, Baton Rouge, LA 70803; Larry J. Livaudais, Jr., MS, LSU FACES Lab, Baton Rouge, LA 70803*

**Learning Overview:** After attending this presentation, attendees will understand the effective role of forensic anthropology in the development of a localized database for long-term unidentified and missing persons cases, commonly referred to as “cold cases.”

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of a collaboration between forensic anthropology, law enforcement, and the public for the successful resolution of cold case unidentified and missing persons investigations at a local level.

Enacted in 2006 by Louisiana state legislative action, the Louisiana Repository for Unidentified and Missing Persons Information program is administered by the Louisiana State University Forensic Anthropology and Computer Enhancement Services (LSU FACES) Laboratory. In addition to the formal Repository database, anthropologists also maintain a comprehensive and fully searchable website that is available to the public ([identifyla.lsu.edu](http://identifyla.lsu.edu)). The website contains biographical and anthropological information for unidentified and missing persons cases within Louisiana that users can search specifically or browse generally.

While national and international databases are available to the public (e.g., NamUs, Doe Network), the additional option of a state-level database allows those who have a more specific inquiry to focus a search that may otherwise seem overwhelming in the larger databases. A goal of the LSU FACES Laboratory is to diligently collect anthropological and biometric data for unidentified remains and biographical information about missing persons from Louisiana law enforcement agencies to augment the Repository. The Repository aims to assist in the resolution of cold cases by employing anthropological methods that link biological data of unidentified remains with the sociocultural knowledge of local communities with missing loved ones.

Attendees will be presented with two examples of Louisiana cold cases, both spanning approximately 35 years, that were resolved with the help of information gained from public use of the Repository website. The first example outlines a case of unidentified human remains discovered in 1981. Anthropological data and forensic images were disseminated to the public until a similarity was detected by a citizen between a facial approximation created at the LSU FACES Laboratory and a missing persons report of a young Michigan woman last heard from in Louisiana. The second example regards the search results of a Tennessee woman curious about the enigmatic background of her deceased mother. Only knowing that her mother was from Louisiana, the woman perused the Repository website and found an image that helped direct her search. While widespread media attention can be helpful in resolving such cases, many unidentified and missing persons cold cases do not get publicized in the national media. For the cases highlighted here, neither of which garnered national attention, local communities and individuals searching with a Louisiana focus were able to use the Repository website, ultimately aiding in the resolution of these cases.

The purpose of this presentation is to discuss methods used by the LSU FACES Laboratory and the Repository for the resolution of unidentified and missing persons cases through the continued devotion of forensic anthropologists, law enforcement agents, coroner’s offices, and local communities to maintain the accessibility and visibility of Louisiana cold cases.

#### **Database, Cold Cases, Forensic Anthropology**



### A22 Introduction of a Mobile Digital Database System for Standardization, Quality Assurance, and Efficiency in Forensic Anthropology Casework

Christine M. Pink, PhD\*, Metropolitan State University of Denver, Denver, CO 80217-3362; Rebecca E. Bria, PhD, University of Minnesota, Minneapolis, MN 55455

**Learning Overview:** The goal of this presentation is to offer a portable and user-friendly Graphic User Interface (GUI) for more efficient and standardized data collection, analysis, peer review, and reporting in forensic anthropology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a mobile digital database system whose widespread implementation would stimulate significant improvement of practitioners' ability to efficiently document field recoveries and laboratory analyses. Additionally, the system would enhance performance in the discipline by allowing for uniform work products, rapid peer review, and consistency in training.

Standardization of the practice of forensic anthropology has been a primary focus within the discipline since it was formalized in the 1970s with the establishment of the (Physical) Anthropology section of the American Academy of Forensic Sciences (AAFS) and the American Board of Forensic Anthropology (ABFA). The bulk of these efforts are concentrated on the improvement of inter-observer error, validation of methods for skeletal analysis, and, more recently, taphonomy and human remains recovery. Less attention has been paid to consistency in the types of data recorded during recoveries and laboratory analyses. This may be due to heterogeneity in training programs for forensic anthropologists. Furthermore, guidelines for agreed-upon best practices in this rapidly advancing discipline were last updated 5-8 years ago.<sup>1</sup> To address this lack of a discipline-wide data collection protocol, this presentation introduces a Mobile Digital Database System (MDDS) for the documentation, management, and analysis of data for forensic anthropology casework.

A systematized digital protocol for documentation would drive uniformity, and thus improve quality of work products across forensic anthropology. The MDDS is a custom application based on an existing and highly successful system designed for archaeological applications using FileMaker Pro® software.<sup>2</sup> The application, which is compatible with a wide range of mobile devices, computers, and the web, establishes a digital workflow with an easy-to-use GUI for selecting from predefined menu options (e.g., method used, character state, soil texture, etc.), entering text notes, and recording other objective data, such as photos, maps, and sketches. Additionally, the user may quickly refer to methodological references within the application. The goals of the MDDS are to: (1) streamline and systemize data entry for improved speed and accuracy; (2) link data as it is collected via a relational database; and (3) link objective data (photos, scores, etc.) with the analyst's interpretations.<sup>2</sup> Data can also be encrypted to maintain the security of sensitive evidentiary information. Because the data entered in these forms are linked in a relational database, the MDDS allows the user to review findings quickly and produce a formatted report. Data may also be auto-populated to existing forms, such as those for submission to the Forensic Data Bank.

This study envisions the MDDS as a practical tool for accessing references, gathering data, recording field and bench notes, and summarizing interpretations. Two or more practitioners using the system can easily and securely share files for the purpose of peer review within or between institutions. The system can also be introduced during training as a way to standardize graduate education in forensic anthropology. Within the application, users may make suggestions and comments that can be considered by a regulating body for updates to the system. Ideally, the MDDS would be reviewed for content by the ABFA and the National Institute of Standards and Technology (NIST) Anthropology Subcommittee at regular intervals.

#### Reference(s):

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2. Bria, R.E. and K.R. DeTore. 2016. Enhanced Archaeological Data Collection and student Learning With a Mobile Relational Database. In E.W. Averett, J.M. Gordon, and D.B. Counts (Editors), *Mobilizing the Past for a Digital Future: The Potential of Digital Archaeology*. Grand Forks, ND: The Digital Press @ The University of North Dakota Grand Forks.

#### Mobile Digital Database, Quality Assurance, Standardization



### A23 A Geospatial Database for Coroner Records: Developing a Collaborative Partnership to Facilitate Undergraduate Research

*Katherine E. Weisensee, PhD\*, Clemson University, Clemson, SC 29634*

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**Learning Overview:** After attending this presentation, attendees will understand the scope of the project and the benefits and difficulties of implementing a similar project at their institution. The goal of this research project focuses on working with the Pickens County Coroner's Office to create a digital database of death investigation records in the county over the past several decades.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by outlining a collaborative research project undertaken at Clemson University. Currently, records are only available in paper files. A digital database allows for the examination of detailed trends related to deaths in the county from both temporal and spatial perspectives.

The Pickens County Office of the Coroner requested assistance in creating a digital database of death records to preserve and archive information, identify inconsistencies within the records, and examine temporal and spatial trends related to deaths in Pickens County over the past several decades. Over the past year, a relational database was developed using the GeoForm application in arcGIS®. Team members enter data from the paper files into the relational database with protocols in place to ensure accuracy and privacy during the data entry process. Following the data entry process or concurrent with the data entry, students will investigate trends in deaths in the county. This research project provides an important service to Pickens County, and it will provide the opportunity for students to work on real-world research projects.

This presentation will outline the structure of the project, including developing a collaborative relationship between the university and county government, recruiting student research assistants, clearing hurdles with the university legal office, and the benefits of using a geospatial database for preserving these types of records. The improved structure of the database in comparison with the previous electronic recordkeeping system will be discussed. The data that is collected as part of this project is associated with other available spatial databases from the county to the level of the consensus block, including factors related to patterns of deaths such as poverty rates, educational attainment, and percent of income spent on alcohol. This presentation will provide attendees with a model of a project that could be implemented on their campus. This type of research project has proven beneficial to the university, county government, and students as well as creating a database that can be used to address several questions of interest to forensic scientists. The availability of this type of data from a rural county in the southeastern United States is a unique data source and provides additional information compared to other datasets from mainly urban areas. Forensic anthropologists frequently serve in undergraduate programs and it may be difficult to incorporate these students into research projects. This research project has proven successful for allowing students to participate in the research process and develop marketable skills in database development and management.

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### A24 Hidden in a Dark Past: The 1887 Thibodaux Massacre of Black Sugarcane Workers

*Davette N. Gadison, MA\*, Tulane University, New Orleans, LA 70118; Mark Rees, PhD, University of Louisiana Lafayette, Lafayette, LA 70503*

**Learning Overview:** After attending this presentation, attendees will understand how hidden histories perpetuate modern-day cycles of structural violence and how applied forensic anthropology can work within communities to break this cycle.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an understanding of the contemporary social relevance of preliminary forensic anthropology casework involving historic human rights violations.

In November of 1887, underpaid Black sugarcane laborers went on strike in Lafourche Parish and surrounding parishes in south Louisiana. The refusal of sugarcane planters to negotiate better wages and working conditions led to violence on November 23, 1887, in Thibodaux, when a group of White vigilantes sought out and gunned down unarmed Black laborers, including their families.<sup>1</sup> The murderers targeted Black neighborhoods. The systematic killings lasted a minimum of two hours. At least 8 individuals were killed, based on medical examiner records, although oral histories suggest 30 to 60 individuals were murdered.<sup>1</sup> According to oral accounts passed down within the community, the remains of the victims were buried in a mass grave on property owned by the city of Thibodaux. By the 1920s, the land was being used as a city dump with an incinerator. Today, the purported location of the mass grave is owned by the historically Black Raymond Stafford American Legion Post No. 513, which is located on the property.

Not only was this event hidden from history by the absence of written records, but stories of the event have also been altered as they have been passed down from one generation to the next.<sup>2</sup> Perceptions of the event in community memory have been split into two starkly different accounts. Modern descendants of the individuals that perpetrated the massacre and others in the community provide a diluted account of the events that took place on November 23, 1887. Their knowledge of the event portrays a mere skirmish in which disgruntled African American sugarcane laborers needed to be taught a lesson. In contrast, descendants of the victims offer a more vivid and horrifically detailed account of a massacre.<sup>2</sup>

The 1887 Thibodaux massacre resulted in the subjugation of Black community members in Thibodaux to various forms of violence and inequity, not unlike the different forms of structural violence that permeate many other communities across the United States. This structural violence reinforces inequalities and represents an injustice perpetuated throughout subsequent generations. Forensic anthropology, cultural anthropology, and forensic archaeology can unearth the truth by uncovering the actual physical evidence of historic events. In bringing to light historic events that have been intentionally hidden and altered, an investigation of the Thibodaux massacre challenges the veracity of dominant history. To date, there has been community outreach and a preliminary archaeological and geophysical investigation of the presumed gravesite. Based on the results of this investigation, recommendations will be made for additional fieldwork, including excavation, community outreach, and descendant interviews.

The forensic scientific community stands to benefit from working with communities in illuminating hidden histories. The 1887 Thibodaux massacre was one of many instances of organized, post-Reconstruction violence in the United States. In addition to racially fueled hatred for formerly enslaved people and their descendants struggling to achieve equal rights, the hidden history of Thibodaux reveals individuals and families striving to attain humane working conditions above starvation wages.

#### Reference(s):

<sup>1</sup> DeSantis J. *The Thibodaux Massacre: Racial Violence and the 1887 Sugar Cane Labor Strike*. Charleston: The History Press, 2016.

<sup>2</sup> DeSantis J. Phone communication with author, April 24, 2018.

#### Human Rights, Massacre, Post-Reconstruction



### A25 The Use and Misuse of Transition Analysis: An Assessment of the Boldsen et al. Age-at-Death Estimation Method

Sara M. Getz, PhD\*, Idaho State University, Pocatello, ID 83209-8005

**Learning Overview:** The goal of this presentation is to increase awareness of the proper use of the Transition Analysis (TA) age-at-death estimation method and its associated software, as well as to present a large-scale assessment of the method's performance across the adult lifespan.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making practitioners aware of some of the most common mistakes made in the application of the TA method and will equip individuals to make informed decisions about the selection of appropriate software parameters for use in forensic contexts.

TA is the only existing adult age-at-death estimation method capable of statistically combining information from features of the cranial sutures, pubic symphyses, and iliac auricular surfaces.<sup>1</sup> Using an associated free computer program, probabilistic age information for independently scored traits and their character states in each skeleton are combined to produce a maximum likelihood estimate of age. This estimate can then be statically adjusted based on prior information about the age structure of the population the individual likely came from. The final result is a probabilistically tailored maximum likelihood point estimate of age with a confidence interval for each individual based on the skeletal features present and the consistency of the age information provided by each trait. Although TA is not as widely used as many traditional methods for the pubic symphysis and auricular surface, it has become increasingly popular in recent years. Increased visibility at professional conferences and in peer-reviewed journals has revealed several common areas of confusion in both the interpretation of trait definitions and the analysis of data using the TA software. In addition to clarifying common points of confusion, this presentation uses a large sample of documented individuals to demonstrate the method's accuracy and precision in each decade of adulthood, discusses the effects of using sex- and ancestry-specific reference samples, and describes the impact of using the archaeological and forensic prior distributions.

A sample of 839 individuals (579 males and 260 females) from five documented skeletal collections—Athens, Maxwell Museum Donated, JCB Grant, UI-Stanford, and St. Bride's Crypt—was evaluated between 2014-2016. Age was estimated for each individual using the latest version of the TA software (ADBOU 2.1.046). Prior to analysis, the TA program requires the selection of sex (male, female) and ancestry (White, Black) categories, as well as the selection of a prior distribution (archaeological, forensic). Selecting unknown for sex, ancestry, or both, results in the use of combined reference sample data; however, the resulting changes in the accuracy and precision of age estimates have not previously been demonstrated. Thus, in this study, age was calculated for each individual using the appropriate sex- and ancestry-specific categories indicated by collection documentation, as well as each of these in combination with an unknown sex or unknown ancestry, respectively.

In addition to a uniform prior—one in which an individual of every age is equally likely to die—the TA program includes two informed priors: a “forensic” distribution based exclusively on homicide data compiled by the Centers for Disease Control and Prevention (CDC) and an “archaeological” distribution derived from 17<sup>th</sup>-century rural Danish parish records that represents normal human mortality. Although the forensic distribution should be used only in forensic cases where homicide is suspected, as a result of its name, this distribution is often indiscriminately applied to all forensic cases. Previously published work has demonstrated the differences between estimates produced using the uniform and archaeological priors are minor until late adulthood (80+ years); however, the effects of the forensic prior have not previously been explored. Therefore, in this study, each sex and ancestry combination was also evaluated using the uniform, forensic, and archaeological distributions to allow for a systematic evaluation of the changes produced in different portions of the adult lifespan.

These analyses demonstrate that use of the archaeological distribution appears to marginally improve accuracy with the uniform distribution. In contrast, inappropriate use of the forensic distribution greatly reduces accuracy, particularly in older age categories. Although the overall performance of TA falls short of what is needed for forensic applications, particularly between 45 and 75 years of age, it represents a substantial improvement over traditional techniques. The method generates individualized age estimates for all of adulthood and, when used correctly, produces relatively high accuracy relative to the precision of its intervals. Additionally, contrary to common expectation, the method produces estimates with similar accuracy, but with greater precision, for the oldest individuals (80+ years) than for individuals in middle age. This effect is seen despite the method's reliance on the cranium and pelvis. Thus, this investigation demonstrates that the transition analysis approach represents a promising avenue for the improvement of adult age estimation if additional features can be identified and integrated into the procedure.

This research was supported by a National Science Foundation Doctoral Dissertation Research Improvement Grant.

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- <sup>1</sup> Boldsen, J.L., Milner, G.R., Konigsberg, L.W., and Wood, J.W. Transition Analysis: A New Method for Estimating Age From Skeletons. In: Hoppa R.D., Vaupel, J.W., editors. *Paleodemography: Age distributions from skeletal samples*. Cambridge, UK: Cambridge University Press, 2002; 73-106.

#### Transition Analysis, Age Estimation, Biological Profile



### A26 A New Method for Adult Skeletal Age Estimation Using Transition Analysis: TA3

*Stephen D. Ousley, PhD\**, Mercyhurst University, Erie, PA 16546; *George R. Milner, PhD*, Pennsylvania State University, University Park, PA 16802; *Jesper L. Boldsen, PhD*, ADBOU, Institute of Forensic Medicine, 5260 Odense S, DENMARK; *Sara M. Getz, PhD*, Idaho State University, Pocatello, ID 83209-8005; *Svenja Weise, PhD*, Institute of Forensic Medicine, Odense, DENMARK

**Learning Overview:** After attending this presentation, attendees will better understand a new system for adult age estimation from the skeleton. The system comprises new trait definitions and scoring procedures, new analytical methods for age estimation, and new software that facilitates the entire process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing a significant gap in best practices in the forensic anthropological approaches to adult age estimation. This method is based on more than 1,600 skeletons from around the world, exhibits little bias in estimating age in individuals approximately 20 to 100 years old, and can use numerous traits from throughout the skeleton.

Accurate and precise age estimates are crucial for identifying skeletal remains. Age intervals assigned to skeletons can rarely provide support for individual identifications. Current age estimation methods for adults are characterized by biased point estimates of age accompanied by prediction intervals that usually span several decades to include virtually all of adulthood. Moreover, open-ended categories such as “50+” are often used because the currently used traits provide no further information after 50 years of age. Clearly, new skeletal traits are needed to better estimate age.

Creating a new system involved defining and refining the new skeletal traits, assessing inter-observer differences, establishing trait age distributions, employing new statistical and machine learning analytical procedures, and developing user-friendly software for scoring traits and estimating age.

Observations were collected from 136 traits, 39 of which are bilateral, from more than 1,600 known-age adult skeletons from four continents to accommodate regional and ancestry-related variation in the aging process. The new system, TA<sup>3</sup>, is an improvement on Transition Analysis, which has focused solely on the pelvic joints and cranial sutures. TA<sup>3</sup> uses many newly defined traits distributed throughout the skeleton. Most traits were scored as binary, either present or absent, usually reflecting additional bone formed over time, such as lipping near joints; judging bones as “light” is an example of scoring bone loss; weights and lengths of certain bones were recorded to quantify bone density; other traits were scored as ordinal traits, depending on the attainment of certain thresholds. Subjective stages defined by numerous features were avoided. The traits that undergo a fast transition from one state to the next during adulthood are especially valuable. Several statistical and machine learning methods were used to combine information from multiple traits to yield valid age estimates and prediction intervals.

The most important general result of this research is that there are numerous traits throughout the skeleton that show changes throughout the human lifespan, enabling more accurate estimates without large open-ended age intervals such as “50+.” The mean correlation for all traits with age is 0.46, and some are far more useful than others. The ordinal traits are often expressed in general age distribution categories of “young” (20 to 40), “middle aged” (40 to 60), or “older” (at least 60). As a result, TA<sup>3</sup> yields far better age estimates throughout adulthood than any other currently used method. For example, a single trait from the femur (fovea margin smooth vs. lipping) shows a more rapid transition and provides a much better age estimate than Suchey-Brooks stage 5 vs. stage 6. The latter finding illustrates the value of defining simple traits from any skeletal area rather than through divining complicated stages by observing many trait states in a few areas.

Other important results include: the cranium appears to have little age-relevant information; subjective “lightness” of bones is valuable in estimating older ages; vertebral and humeral traits are very informative; many traits show high intercorrelations, and bilateral traits are, for the most part, very highly correlated; marked asymmetry seems restricted to one trait from the ulna.

The analytical methods are still being refined but results so far show age estimates with 95% prediction intervals of as small as +/-10 years, and most importantly, with little to no bias in age estimation between 20 and 100 and no plateau in age estimates. Using more traits from different bones provides more accurate estimates. Also, because TA<sup>3</sup> can use bony features distributed throughout the body, meaningful age estimates are possible for incomplete skeletons, which often is essential in forensic investigations.

This project was funded through a National Institute of Justice (NIJ) award.

#### Biological Profile, Skeletal Age Estimation, Transition Analysis





### A27 New Solutions for Old Problems? Examining Machine Learning as a Strategy for Age-at-Death Estimation

Melissa Ann Brown, MA\*, Western Michigan University, Kalamazoo, MI 49006; Dillon G. Daudert, BS, Kalamazoo, MI 49006; James Jenkins, BS, Galesburg, MI 49053

**Learning Overview:** The purpose of this presentation is to provide the forensic science community with a review of machine learning computational approaches, while assessing its utility to age-at-death estimation. After attending this presentation, attendees will better understand the essential nature of machine learning and the impediments to implementing such technology in applied contexts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explicating the challenges of applying machine learning, a novel computational approach, to complex skeletal analysis, while providing guidance on best practices for future research.

This research examines the efficacy of a machine learning approach known as a Convolutional Neural Network (CNN) to the estimation of age-at-death by analysis of 3D scans of the pubic symphysis. Machine learning, a subfield of computer science, is a process that allows computers to execute decision-making tasks without specific human supervision. CNNs are especially successful in tasks relating to image categorization. CNNs have demonstrated competency greater than trained professionals in medical contexts.<sup>1</sup> A preliminary evaluation of a CNN's capacity to accurately assess age at death relative to trained osteologists was conducted to determine if this technology is potentially useful in forensic evaluation.

A CNN was built using the TensorFlow™ v1.7 open-source software library. To allow the CNN to learn associations between pubic symphysis morphology and age, it was provided a training batch of 3D images of the pubic symphysis from decedents of known age at death. Training images were created using the NextEngine® 3D Desktop Scanner. Scans were collected from individuals in the Hartnett-Fulginiti Collection of modern American pubic symphyses. The left aspect of the pubic symphysis of  $n=292$  male individuals, aged 18–99 years, were scanned, processed, and provided to the CNN. After training, the CNN was tested on a novel selection of  $n=16$  individuals chosen to proportionally represent the age distribution of the training sample. To provide a litmus for CNN performance, osteologists were recruited and asked to assess the same test sample. Volunteers, evaluating real bone, were asked to assign individuals to a Suchey-Brooks phase score.

Currently, results indicate that CNNs are not a viable approach to age-at-death estimation. CNN-generated ages are produced by a linear regression function output method which ubiquitously overestimates age in younger individuals, while underestimating the age of the elderly. Further, the CNN is prone to error where humans are not, such as misinterpreting postmortem damage that volunteers can easily recognize. The CNN does excel at assigning accurate age-at-death estimates for middle-aged individuals, which are accurate within ten years. However, because of the CNN's habit of producing middling values, these results must be regarded cautiously.

The trend toward middle-age value outputs is likely reflective of population biases in the training set, wherein middle-aged individuals account for more than half of all training data. Evidence that demonstrates CNN learning may be found in cases where both the CNN and observers anomalously misclassified individuals in accordance to non-normative morphology, such as marked overestimation of age in a 21-year-old displaying unusually advanced skeletal changes. This suggests that further training of the CNN with a deliberately biased data set to compensate for deficiencies in experience among the young and elderly will improve output results. However, meeting the training demands of a CNN, which often require many thousands of samples for high accuracy, may not be feasible given the nature of the data required. Further, natural morphological diversity may preclude refining age estimates much further beyond current methods. Such challenges, which are unique to anthropology, may preclude integrating machine learning approaches to applied osteology.

#### Reference(s):

1. Esteva, Andre, Brett Kuprel, Roberto A. Novoa, Justin Ko, Susan M. Swetter, Helen M. Blau, and Sebastian Thrun. Dermatologist-Level Classification of Skin Cancer with Deep Neural Networks. *Nature*. 542, no. 7639 (Feb 2017): 115–18.

#### Machine Learning, Age-At-Death Estimation, Computational Anthropology



### A28 Bone Mineral Density (BMD) as an Indicator of Age at Death in Forensic Anthropology: A Test of DXAGE

Jonathan D. Bethard, PhD\*, University of South Florida, Tampa, FL 33620-8100; Jacqueline M. Berger, MS, University of South Florida, Tampa, FL 33620-7200; Justin R. Maiers, MS, University of South Florida, Tampa, FL 33647; Ann H. Ross, PhD, North Carolina State University, Raleigh, NC 27695-7614

**Learning Overview:** After attending this presentation, attendees will understand how BMD can be utilized as an age-at-death indicator in forensic anthropological contexts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that forensic anthropologists may be able to utilize easily obtainable and quantitative BMD parameters for estimating age at death.

Estimating the age at death of individuals represented only by skeletonized human remains is a fundamental aspect of forensic anthropological casework. Traditionally, forensic anthropologists utilize macroscopic features of the skeleton to arrive at these age-at-death estimates; however, in many instances, the most common age indicators are absent or can only be used to arrive at broad, forensically useless age estimates. In addition, forensic anthropologists have difficulty accurately and precisely estimating age at death in individuals older than 50 years due to variation in the aging process. To rectify these shortcomings, several researchers have proposed that BMD is a useful predictor of age at death in forensic anthropological contexts. Given the well-known relationship between increasing age and decreasing BMD, scholars have hypothesized that age-at-death estimates can be generated from the analysis of BMD. Moreover, BMD data are quantitatively generated from bone densitometry scans and remove issues related to observer subjectivity.

Navega et al. utilized BMD data from femora of 100 individuals drawn from the Coimbra Identified Skeletal Collection in Coimbra, Portugal.<sup>1</sup> Navega and coworkers utilized artificial neural networks and created a user-friendly, web-based interface called DXAGE. DXAGE permits forensic anthropologists to generate age-at-death estimates from one or more BMD variables. A predicted age, as well as a minimum and maximum age-at-death estimate, are also calculated by DXAGE.<sup>1</sup> One of the advantages of a neural network is that it can efficiently model difference response layers, while a major limitation is that the results are not easily interpretable as there is not a direct path from x to y variables as in regular regression. Thus, the purpose of this study was to test DXAGE by utilizing BMD data from the National Health and Nutrition Examination Survey (NHANES).

A subset of NHANES femoral BMD data from 470 females over the age of 20 years was utilized. Using the NHANES BMD data, age was calculated in DXAGE and these predicted ages were compared to the known ages from the NHANES dataset. The mean difference between predicted and actual ages was assessed with the Matched Pair platform in the statistical package JMP 13.1, which compares row-by-row differences between two response columns (i.e., predicted age and actual age) using a paired *t*-test. In addition, comparisons were made within age decades to explore the decades with the greatest difference. Results demonstrate that there is a weak correlation between predicted and actual age ( $r=0.47$ ). Results also show that there is a significant difference between predicted age and actual age in the overall model ( $\text{Prob} > |t| < .0001$ ; Mean Difference F Ratio 34.82) and show that on average DXAGE under ages individuals by 7 years (Mean Difference = -7.2 years). In the youngest age category (20–29 years), the Mean Difference is 7.47 years. In other words, in this younger age cohort, DXAGE on average over ages by 7.5 years. The age category with the least mean difference is 30–39 (Mean Difference=0.65 years). DXAGE under ages individuals in the remaining age categories: by 5.3 years in the 40–49 age group; by 9.7 years in the 50–59 age group; by 15.45 years in the 60–69 age group; by 24.44 years in the 70–79 age group; and by 23.1 years in the 80+ age group. Overall, this study demonstrates that BMD data may be of use to forensic anthropologists for generating age-at-death estimates.

#### Reference(s):

1. Navega D., Coelho J.D., Cunha E., Curate F. DXAGE: A New Method for Age At Death Estimation Based on Femoral Bone Mineral Density and Artificial Neural Networks. *Journal of Forensic Sciences*. 2018;63(2):497-503.

#### Age-At-Death Estimation, Bone Mineral Density, DXAGE



### A29 Age Estimation on Two Mediterranean Populations Using Rib Histomorphology

*Julieta Gomez Garcia-Donas\**, University of Edinburgh, Edinburgh, Scotland EH8 9AG, UNITED KINGDOM; *Andrea Bonicelli*, MSc, University of Edinburgh, Edinburgh EH8 9AG, UNITED KINGDOM; *Caroline Lill*, MSc, University of Edinburgh, Edinburgh, Scotland EH8 9AG, UNITED KINGDOM; *Robert R. Paine*, PhD, Sociology & Anthropology; FS Program Director, Lubbock, TX 79409; *Elena Kranioti*, PhD, Edinburgh Unit for Forensic Anthropology, Crete, GREECE

**Learning Overview:** After attending this presentation, attendees will better understand histological methods, both protocols and anthropological applications. A discussion on bone remodeling and its use for estimating age at death will provide a comprehensive understanding of bone at the histological level.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a deeper understanding of the microscopic approach, which is not a common practice but is the only option for the identification of fragmented remains.

One of the crucial steps for the creation of the biological profile of an individual is the estimation of age at death. The choice of the method depends on the nature of the remains, the equipment available and the expertise of the forensic anthropologist, among others. In cases of very fragmented remains, microscopic methods remain one of the only approaches that can be applied. This study presents the results obtained from a histological analysis of rib thin-sections from two Mediterranean populations.

The sample consists of 88 standard ribs from two Modern samples (Cretan and Greek-Cypriot Collections, N=88, Mean age=60, SD=17.90). The costal elements were processed histologically according to standard protocols. Thirteen variables (both qualitative and quantitative parameters) were assessed. Technical Error Measurement (TEM) analysis was performed to test the repeatability of the histological parameters. A validation study was performed by applying four existing microscopic methods to verify whether a formula is required for the sample at hand. The correlation between the variables and age was examined through different statistical approaches. The results were used for the generation of linear models using the whole sample and the sample divided by sexes and populations.

Intra- and inter-observer errors demonstrated that the variables presented different levels of agreement. Three out of four of the methods exhibited a systematic underestimation of the individuals producing high error rates. Most of the variables demonstrated a significant correlation with age and some differences were observed between sexes and samples (Cretans and Greek-Cypriots). A total of 41 models were generated and 12 were selected as the most accurate with a standard error of the estimate ranging from 12 to 8 years. A comparison between the Mediterranean samples and other populations exhibited different patterns on bone remodeling, with the Cretan sample having the lowest Osteon Population Density (OPD) among others.

This research demonstrates the use of quantitative histology for the estimation of age at death, producing accuracy rates similar to those provided by macroscopic methods. The poor results obtained by the existing histological formulas confirmed the need for a population-specific equation for Cretans and Cypriots. Possible intrinsic and extrinsic factors may be the cause of the observed inter-population variation, with differences in nutrition and genetics being considered as the potential causes. Thus, interesting patterns on remodeling rates provided a new insight on bone histological parameters for the sample under study.

#### **Bone Histology, Age Estimation, Mediterranean Populations**



### A30 Utilizing Osteon Volume for Histological Age-at-Death Estimation

*Suzanna Michener, MSc\*, Simon Fraser University, Burnaby, BC V3Z 0E3, CANADA; Lynne S. Bell, PhD, Simon Fraser University, Burnaby, BC V5A 1S6, CANADA; David Swanlund, BA, Simon Fraser University, Burnaby, BC V5A1S6, CANADA; Nadine Schuurman, PhD, Simon Fraser University, Burnaby, BC V5A1S6, CANADA*

**Learning Overview:** After attending this presentation, attendees will appreciate the utility of incorporating Geographic Information Systems (GIS) technology to visualize and analyze the spatial distribution of histological structures, as well as to quantify and measure the size and type of these structures in bone in three dimensions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving collective understanding of both osteonal volumetric calculations and how such information can be used to improve human age estimation.

Histological examination of remodeling events in cortical bone has been used to estimate adult age at death if appropriate macroscopic elements are damaged or absent. There are several approaches that have examined different skeletal elements, variable histological variables, and diverse regions of interest. What is common to all these methods is that a single transverse section is utilized, thus viewed in two dimensions. Osteons and associated bony remodeling events are 3D, dynamic structures. Osteonal shape may be round to elliptical in cross section and 200–250 microns in diameter; cylindrical in shape and one to ten millimeters in length; and oriented at 11–17 degrees off the longitudinal z-axis. None of these known 3D features have been incorporated into histological age estimation methods. Incorporating osteonal features, such as volume, into age regression equations, may improve the accuracy and replicability of age estimation formulae.

The current study employed a GIS-based analytical approach to digitally map, quantify, and connect remodeling events on three serial cross-sections, providing a 3D, volumetric perspective of bone remodeling. To develop the method, vertical alignment grooves were cut into the cortex of an adult human femoral midshaft to maintain multi-planar spatial orientation. Three serial thin-sections of approximately 70 microns were subsequently cut; they were separated by 300 microns due to blade thickness, resulting in an overall z-axis dimension of approximately 800 microns. Each cross-section was photographed in its entirety under circularly polarized light and composited using panorama-stitching software. Using georeferencing tools, the three cross-sections were aligned as overlays in arcGIS® v10.2. Intact and fragmentary osteons were manually outlined using polygon feature classes; these histological units were then connected across the cross-sections utilizing buffer and join processes. The centroid was identified for each cross-section, permitting the overlay of a quadrant and octant system. This allowed comparison of histological variables between quadrants both within and between cross-sections.

One thousand osteons were outlined using polygon feature class layers for each cross-section and a random sample of 30 connecting osteons were selected from eight octants (anterior, medial, posterior, lateral, anteromedial, posteromedial, anterolateral, and anteromedial) for comparative analyses. The area of the osteons was compared between quadrants and across layers using Analysis of Variance (ANOVA) statistical tests. Osteonal area was found to be smaller in the anterior portion of the femoral cross-section, likely due to faster remodeling from increased strain. Osteon area was also compared within quadrants between cross-sections. There were statistically significant ( $p < 0.05$ ) differences within the same quadrant of different transverse sectional layers. These results suggest that osteon area may not be a reliable indicator for histological analyses, as the average area of osteons differs between layers a mere 300 microns apart. Utilizing truncated cone geometric functions, the volume of the 240 connected osteons was interpolated and compared between quadrants. Osteon volume was significantly different between the anterior and posterior portions of the transverse section; however, there were no significant differences in osteon volume between quadrants of the same portions. That is, anterior, anterolateral, and anteromedial did not differ from each other, and nor did the posterior, posterolateral, and posteromedial quadrants differ from each other. These results indicate that region of interest may matter less when utilizing volumetric interpolations for histological variables. Volumetric calculations may be more robust and consistent across serial transverse sections and, if incorporated into age-at-death estimation equations, may serve to improve the accuracy and reproducibility of such approaches.

#### Age Estimation, Skeletal Histology, Geographic Information Systems



### A31 Humanitarian Forensics: Applications Today and Emerging Challenges

*Oran Finegan, MSc\*, International Committee of the Red Cross, Geneva, SWITZERLAND; Pierre M.M. Guyomarc'h, PhD, International Committee of the Red Cross, Geneva, GVA 1202, SWITZERLAND*

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**Learning Overview:** After attending this presentation, attendees will better understand the application of forensic science in the humanitarian sphere and how the evolution of conflict is changing the way we think about how forensic science is applied.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the need to rethink the approach that has been taken over past decades to the application of forensics in conflict.

The last (approximately) 40 years have seen a growing role for, and application of, the diverse range of fields of forensic science to the humanitarian and human rights field. This has been particularly true for the field of forensic anthropology. The 1980s saw the emergence of this with the work of teams such as the Argentine Forensic Anthropology (EAAF) team, spawning a number of similar groups throughout Latin America.

Following its emergence, growing advancements in forensics, such as the fields of forensic anthropology and genetics, saw the large-scale application of forensics in regions such as the Balkans, following the conflicts there toward the end of the 20<sup>th</sup> century. The experiences of the Balkans brought some balance to the understanding of what was possible, in theory and in practice, when it came to the use of forensics to both assist in the pursuit of justice (e.g., International Crimes Tribunal for the Former Yugoslavia) and the humanitarian need for the identification of the many persons who remained unaccounted for at the end of the conflicts. While great advances were made in forensics throughout the world due to this work during the 1990s and early 2000s, particularly when it came to understanding the recovery and analysis of large-scale assemblages of human remains (for which forensic anthropology played a growingly invaluable role), it was also clear that there were limits as to what could be achieved. Hopes to see the identification of all those who were unaccounted for were soon tempered by the practical realities of situations encountered.

Nearly 20 years later, we have a much better understanding of forensic science and how it can be applied in both conflicts and disasters. There has been a growing shift away from the large post-conflict and post-disaster response, to better understanding how forensics, and forensic anthropology, can assist in both preparations for possible disasters and also better support efforts to identify persons who become unaccounted for because of conflict by engaging prior to and during conflict itself. In many ways this is the emergence of a new era in the application of forensics to humanitarian and human rights issues. The evolution of conflict today, with a more protracted and urbanized character, means we need to further rethink how forensics can continue to be relevant and applicable. Recognizing the need for strengthening global forensic science and supporting the empowerment of domestic forensic structures, where present, is fundamentally important. There continues to be very weak structures when it comes to the field of forensic anthropology and much needs to be accomplished to understand how to best address this. The experiences of organizations such as the International Committee of the Red Cross, which today globally has the largest forensic team working in the humanitarian sphere, is demonstrating that the delivery of ad hoc short courses in forensic anthropology in many ways risks undermining the discipline itself, if the correct target audience is not identified. The use of data, in particular, ideas around big data, and predictability models to help determine the location of persons unaccounted for will also be important areas of study.

Ultimately, a better understanding of conflict, disaster, and the cultural, religious, and social needs of the affected population will be key if the emerging field of humanitarian forensics is to continue to play a growing and valuable role in the 21<sup>st</sup> century.

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#### Humanitarian Forensics, Armed Conflict, Emergency Response



### A32 After Atrocity: Why Wouldn't You Investigate?

*Dawnie W. Steadman, PhD\**, University of Tennessee, Knoxville, TN 37996; *Hugh H. Tuller, MA*, Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI 96853-5530; *Julia Hanebrink, MA*, Rhodes College, Memphis, TN 38112; *Jaymelee Kim, PhD*, University of Findlay, Findlay, OH 45840-3653; *Tricia Hepner, PhD*, University of Tennessee - Dept of Anthropology, Knoxville, TN 37996

**Learning Overview:** After attending this presentation, attendees will understand the underestimated complexity of community needs, aspirations, and perceptions concerning post-conflict humanitarian forensic actions for mass graves.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring the use of ethnography to assess the potential of forensic anthropology to support survivor needs and transitional justice processes in northern Uganda.

The two-decade-long war (1986–2006) between the Lord's Resistance Army (LRA) and the Government of Uganda (GoU) resulted in the displacement of 90% of northern Ugandans, the abduction of tens of thousands of children who were killed or forced to become child soldiers, and the murders of possibly tens of thousands of civilians. One enduring legacy of the conflict is the landscape of discarded bodies; some left on the surface to decay, others hastily buried, and more placed in mass graves, often far from their villages, or in Internal Displacement (IDP) camps where they had been forcibly displaced by the government.

The lack of health facilities in the region preclude the use of dental and medical records or fingerprints for identification, leaving DNA as the prime modality for identification. Thus, forensic scientists may quickly deduce the most logical approach to the identification problem in Uganda consists of anthropological intervention and construction of a DNA database for the missing. Often, it is assumed that the use of forensic tools is desired, if not prioritized, and positively viewed by affected communities. However, Northern Ugandans have little experience with DNA or other forensic science fields, no trust in the government, little faith in foreign Non-Governmental Organizations (NGOs), no evidentiary concerns for the graves, and a long list of apprehensions (e.g., security, education, land rights, daily subsistence) beyond that of the missing. Moreover, survivors remain troubled by the graves as many believe that the spirits of the dead maintain agency among the living, causing maladies and disturbing hauntings, making it possible that forensic exhumations might cause greater psychosocial harm than good.

Since 2012, a team based at the University of Tennessee has been conducting ethnographic work in northern Uganda to assess the various needs of the communities related to forensic intervention. The results in this presentation are centered on more than 160 in-depth, semi-structured interviews with individuals and small focus groups in six villages in northern Uganda, as well as interviews with government personnel, NGOs, and pathologists. While forensic investigation following atrocity is usually portrayed as a binary decision (investigate or not), a cultural anthropological approach demonstrates that the reality on the ground is much more complicated. While many communities are supportive of forensic intervention and do wish to find their loved ones, others may view investigations as unnecessary due to religious beliefs or feel that the presence of mass graves and their associated monuments supports community claims for reparations and view an identification process as potentially disruptive to these claims. Furthermore, as forensic investigation may become a source of secondary traumatization for families, medicolegal evidentiary analysis is sometimes viewed as less desirable than non-invasive, more culturally salient measures such as ceremonial and/or religious remedies. The findings illustrate how the ethnographic research process produces intrinsically valuable and forensically relevant insights, regardless of whether excavations occur.

**Reference(s):**

<sup>1</sup> Corder S., and M. Tidball-Binz. 2017. Humanitarian Forensic Action—Its Origins and Future. *Forensic Science International*. 279:65-71.

**Forensic Anthropology, Human Rights Investigations, Cultural Anthropology**



**A33 WITHDRAWN**



**A34 WITHDRAWN**





### A35 Capacity Building of Forensic Scientists

*Elizabeth A. DiGangi, PhD\*, Department of Anthropology, Binghamton, NY 13902; Jonathan D. Bethard, PhD\*, University of South Florida, Tampa, FL 33620-8100*

**Learning Overview:** After attending this presentation, attendees will understand the methods and best practices undertaken for capacity building of forensic scientists, primarily forensic anthropologists, in diverse locations such as Algeria, Colombia, Mexico, and Georgia.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the importance of using an anthropological perspective when training scientists in countries outside of the United States. Cultural sensitivity and awareness are required to ensure that scientific learning objectives are imparted and ideally incorporated into practice.

Forensic scientists trained in industrialized countries often have the luxury of having been exposed to a first-class education, with little expense spared to provide for their training, both theoretical and practical. In the case of forensic anthropology, those of us working in the United States or Canadian contexts in particular (with a few notable exceptions), are able to use that education primarily for isolated missing persons or homicide cases. Ironically, colleagues who work in countries where missing or disappeared persons issues are systemic and widespread have often not had the privilege of a similar education in forensic anthropology. This is due to several factors that are country-specific, but, most generally, it is because forensic anthropology was born as a discipline in the United States and remains one of the youngest forensic sciences, having only been professionalized four decades ago.

As a result, forensic anthropologists or other forensic scientists and law enforcement professionals are increasingly being asked to travel to countries with a human identification issue stemming from dozens to thousands of missing or disappeared persons and train the local anthropologists or forensic scientists. Perhaps the most famous example of this in action would be the tireless efforts of the Argentinean Forensic Anthropology Team, who have traveled to numerous countries to train local practitioners and/or do forensic anthropological work themselves. This presentation discusses the experiences of working in several different countries with the goal of capacity building local scientists and the conclusions regarding best practices.

We have trained forensic scientists and law enforcement personnel from a variety of disciplines but most often forensic anthropologists in Algeria, Colombia, Georgia, and Mexico. The first rule of working with colleagues in another country is also the first rule of applied anthropology, that is, cultural relativism. Further, we must realize the need to be accepting of cultural differences when it comes to relevant issues such as treatment of the dead. Second, we must educate ourselves about the country's context in terms of what the issues have been that led to a need for human identification and what training the forensic scientists already have. It can be tempting to think that we would not be there unless the need existed; however, we must recognize our privilege at having been invited to share our knowledge with local scientists and realize that we are there to learn from them, too.

Further, we must understand that depending on a country's particular socio-historical context, our colleagues may not be able to or willing to implement all our recommendations. We must therefore be able to fully understand the context and, importantly, tailor our advice based on that context. We ask ourselves, "What is an ideal situation to practice forensic archaeology and forensic anthropology—and what practices can be altered in a way that scientific integrity is not harmed when the situation is not ideal?" Non-ideal situations are the rule rather than the exception and range from limitations related to funding to infrastructure to socio-political contexts prohibiting the personnel from conducting anthropological work at all.

Ultimately, we are training our colleagues to practice their own forensic anthropology, and we must do this in a way that is both palatable to them culturally and realistic given each particular context. Finally, we must contemplate if any ethical issues exist. We have worked for both governmental and non-governmental organizations and working for the former may raise certain questions about its agenda and our contribution to that agenda.

#### Capacity Building, Training, International Collaboration



### A36 Assessing the Role of Churches in Missing Migrant Identifications

*Richard Phillips, BA\*, Duke University, Durham, NC 27708; Sara H. Katsanis, Duke University, Durham, NC 27705*

**Learning Overview:** After attending this presentation, attendees will better understand the role of churches in preventing migrant deaths and in assisting in missing migrant identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the interests and contributions of churches as stakeholders in mitigating the missing migrant crisis in some of the deadliest counties along the United States-Mexico border.

Militarization of the United States-Mexico border in recent years has pushed an increasing number of undocumented migrants from crossing at urban centers to using more remote and dangerous areas, particularly in Texas. The result is an increase in deaths, robbery, extortion, rape, and human trafficking. Every year, hundreds of unidentified human remains are discovered in private, remote ranches in South Texas. For many years, the lack of resources and willpower to provide autopsies and investigations into migrants' deaths means that many were buried hastily, without DNA collection or documentation of burial sites.

Churches historically have assisted migrants during their journeys: providing them food, clothing, and refuge; connecting them with legal services; offering advice on how to best continue to their destination; addressing their spiritual needs and those of affected families; and facilitating burials. In South Texas, Christian churches have an active presence on the United States-Mexico border, particularly the Catholic, United Methodist, and Evangelical denominations. To date, the role of these faith-based communities in working with migrant families is not clearly delineated or understood among policymakers and Non-Governmental Organizations (NGOs). To gather some insight into the perspectives of church leaders on the missing migrant crisis and investigations in Texas, a survey was conducted of a sampling of South Texas-area churches. The purpose of this study was to: (1) gauge the attitudes of Christian church leaders and their congregations toward migrant issues; (2) document their roles in assisting undocumented migrants; and (3) discover potential unidentified remains burial sites.

The area of South Texas includes eight counties (Brooks, Cameron, Hidalgo, Jim Hogg, Kenedy, Starr, Willacy, and Zapata), encompassing 438 churches (both Catholic and Protestant) identified by this study as operating within this region. A database of churches was compiled from churchfinder.com, Google® maps, and each Christian denomination's online directory, and a custom map reflecting this database was created. Using this database, a strategy was designed to gather data from a representative sampling of these churches. This strategy enabled deep questioning from a broad swath of church types. The study assessed parking lot areas to differentiate large and small congregations, sampled Catholic, Mainline Protestant, and Evangelical Protestant churches, and selected for churches in rural and urban areas. This approach enabled the study to parse discrete differences among congregations and regions along the border. The survey questionnaire included questions to assess each church's demographics, cemetery services, social outreach, perspectives (on immigration and other socio-political issues), and theological leanings. These data will inform future studies to gather in-depth data among the various congregations.

The study was approved by Duke University Institutional Review Board (IRB) to proceed, yet additional permissions were requested from leaders in certain churches, denominations, and regions. Some of the requests conflicted with IRB confidentiality requirements, while others required months-long approval processes from denominational leadership. This limited the ability to assess certain groups. These challenges in gaining permissions are important to acknowledge for future studies, but also to recognize the potential barriers to cooperation for migrant death investigations.

In preliminary analysis of the participants' results, the study has found that approximately one-third of the sampled churches have been approached for help in finding missing migrants. Most churches have reported being involved with undocumented migrants, with ~80% indicating that it was important to provide outreach to undocumented migrants. Approximately one-fourth of the sampled churches had hosted a funeral for an unidentified person or a memorial for a missing migrant. The results also indicated a potential difference in perspectives among the parishioners in comparison to church leaders on key issues on immigration (such as extending authority of the United States Immigration and Customs Enforcement to local/regional law enforcement).

These results suggest that Christian churches in South Texas act as mechanisms for American communities to interact with and support migrants in need. Their utility should be considered in any future inter-agency efforts to investigate migrant deaths.

#### Missing Migrants, Stakeholders, Churches



## A37 The Assessment of Jugular Growth Plate (JGP) Ossification for Age Estimation

Brittany S. Walter, PhD\*, Defense POW/MIA Accounting Agency Laboratory, Offutt Air Force Base, NE 68113; Katherine Skorpinski, PhD, Defense POW/MIA Accounting Agency, Offutt Air Force Base, NE 68113

**Learning Overview:** After attending this presentation, attendees will understand the accuracy and utility of JGP ossification as a skeletal indicator for estimating age at death of human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how JGP ossification is not a reliable or useful skeletal indicator for age estimation and by establishing that jugular canal size may influence JGP ossification.

When estimating age from human remains, cranial skeletal indicators of age are useful when postcranial age indicators are absent or damaged. These methods typically provide large/open-ended age intervals for adults (e.g., cranial sutures and third molar development). Previous studies have suggested the ossification of the JGP, located on the posterolateral wall of the jugular canal, as a possible age indicator; however, these studies are limited and contradictory.

Anatomical literature cites JGP ossification to begin by 25 years of age, though the validity of this assertion was not tested until the 1990s. Two published studies have evaluated age at death using JGP ossification.<sup>1,2</sup> Maat and Mastwijk assessed JGP ossification of 98 skulls by removing part of the occipital to determine if ossification was present.<sup>2</sup> In their sample, there was no evidence of ossification until 22 years of age, and all males exhibited bilateral JGP ossification by 36 years of age. Hershkovitz et al. used radiographs and macroscopic analysis of 1,869 crania to assess age of JGP ossification.<sup>1</sup> This study concluded that if there is no evidence of ossification, the individual is likely less than 20 years of age; further, it found no effect of laterality and observed unossified JGPs in individuals above 70 years of age.

The purpose of this study is to assess the validity of previous studies that have quantified JGP ossification patterns for age estimation using an independent sample.<sup>1,2</sup> Crania from individuals of known age ( $n=65$ ) were visually examined for ossification of the JGP. All crania were from identified American World War II (WWII) service members from the USS *Oklahoma* loss at Pearl Harbor on December 7, 1941. The sample population comprised White, Black, and Asian males aged 18.7–48.5 years at death. Ossification of the JGP was independently scored in the blind for each side as “unossified” if there was no evidence of ossification or “ossified” if any evidence of ossification was present.

In the study sample, the youngest age exhibiting JGP ossification is 18.7 years, the youngest age in this sample; and the oldest age at which the JGPs remained bilaterally unossified is 34.3 years. Individuals 44.7 years of age and older ( $n=2$ ) exhibit bilateral ossification. Age estimation following Hershkovitz et al. (i.e., no evidence of ossification for either side indicates <20 years of age) provides a 19.0% correct classification rate, and following Maat and Mastwijk (i.e., bilateral ossification indicates at least 36 years of age) provides an 18.5% correct classification rate.<sup>1,2</sup> Contrary to Hershkovitz et al., laterality had a significant effect on JGP ossification, with a higher frequency of unossified JGPs on the right side compared to the left side ( $p<0.00$ ,  $\chi^2=17.82$ ).<sup>1</sup> Post hoc analyses suggest that this may be an effect of jugular foramen size, which was observed to be larger on the right side in the sample ( $p=0.03$ ,  $\chi^2=4.57$ ). Additionally, inter-observer analyses indicate substantial agreement between the authors ( $K=0.78$ ,  $p<0.00$ ).

To conclude, though inter-observer agreement is substantial, the use of JGP ossification to estimate age is not recommended due to low correct classification rates and the effect of laterality. The youngest individuals in the study sample exhibit both bilaterally ossified and unossified JGPs, indicating that additional data from younger individuals is needed to establish the age of earliest occurrence of ossification. Though bilateral ossification occurs by 44.7 years of age ( $n=2$ ), the limited age range of this sample may not capture ossification status at older ages. Previous observations of unossified JGPs in individuals over the age of 70 suggest that JGP ossification is an unreliable age indicator for older age groups.<sup>1</sup> Additionally, both observers found that on several occasions, the view of portions of the JGP was obscured by surrounding bone, inhibiting scores to be observed and further limiting the utility of this method.

### Reference(s):

1. Hershkovitz I., Latimer B., Dutour O., Jellema L.M., Wish-Baratz S., Rothschild C., and Rothschild B.M. The elusive petrooccipital articulation. *Am J of Phys Anthropol.* 1997; 103:365-373.
2. Maat G.J.R. and Mastwijk R.W. Fusion status of the jugular growth plate: An aid for age at death determination. *Int J of Osteoarchaeol.* 1995; 5:163-167.

### Age Estimation, Jugular Growth Plate, Biological Profile



### A38 Age-at-Death Estimation From the Auricular Surface of the Ilium: A Comparison of Two Methods

Andrea Ost, MS\*, Erie, PA 16507; Luis L. Cabo, MS, Mercyhurst University, Erie, PA 16546

**Learning Overview:** After attending this presentation, attendees will better understand two different methods for age-at-death estimation from the auricular surface and how their scoring criteria can influence the obtained age estimates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by further demonstrating the utility of the auricular surface as a skeletal age marker, particular in aging older individuals.

Accurate age-at-death estimation (*aging*, hereafter) is important for both paleodemographic studies and forensic casework. The most popular aging methods use the cranial sutures, sternal rib ends, pubic symphysis, and auricular surface of the ilium. Although these conventional methods are well-validated, all of them share a common problem in the lack of precision of their age estimates for older individuals (generally over 60 years).

Two auricular surface component methods have shown promising results in improving the estimates for older individuals. The first method, developed in 2005 by Igarashi and colleagues on a Japanese sample ( $N=700$ ), scores the auricular surface on a binary scale for the presence/absence of 13 traits.<sup>1</sup> Igarashi et al. includes separate equations for males and females, which is discordant with methods popularly employed in the United States. The present study compares Igarashi et al. to Transition Analysis, developed in 2002 by Boldsen et al.<sup>2</sup> Transition Analysis uses Bayesian conditional probabilities to combine multiple skeletal age indicators, including the auricular surface, into an age estimate that can be performed with fragmentary remains. This study tested the utility of these two methods on a sample of 400 individuals, aged 16–93 years, from the Hamann-Todd Collection.

Accuracies were compared between groups through goodness-of-fit tests, and Spearman's rank-order correlation was utilized to assess the correlation with age of both overall method and individual trait scores. Igarashi et al. tended to underage both males and females, with the latter significantly more underaged.<sup>2</sup> The development of sex-specific equations with this method did not markedly increase accuracy. Although Igarashi et al. does not appear to accurately estimate age at death, some of its unique traits do show a high correlation with age, indicating that these traits ought to be reexamined. Transition Analysis, despite exhibiting disparate distributions in the middle age ranges, displayed unprecedentedly high accuracy rates in the young and, particularly, the old age classes. Furthermore, although the auricular surface is not meant to be used in isolation with Transition Analysis, the documented ages of the individuals fell into the range provided by the method 87.5% of the time. When combined with other skeletal indicators that could have more utility in the middle age ranges (e.g., pubic symphysis and cranial sutures), this method may provide both precise and accurate results for all age classes. In conclusion, this research demonstrates that, when analyzed according to scorable changes, the auricular surface shows high potential for aging older individuals.

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2. Igarashi, Yuriko, Kagumi Uesu, Tetsuaki Wakebe, and Eisaku Kanazawa. New Method for Estimation of Adult Skeletal Age at Death from the Morphology of the Auricular Surface of the Ilium. *American Journal of Physical Anthropology*. 128 (2005): 324-339.

#### Age Estimation, Auricular Surface, Transition Analysis



### A39 A New Version Release of 3D-ID Geometric Morphometric Classification of Crania for Forensic Scientists

Ann H. Ross, PhD\*, North Carolina State University, Raleigh, NC 27695-7614; Dennis E. Slice, PhD, Florida State University, Tallahassee, FL 32306-4120; Desiré Brits, PhD, University of the Witwatersrand, Johannesburg, Gauteng 2193, SOUTH AFRICA; Tamara L. Lottering, BSc, University of the Witwatersrand, Johannesburg, Gauteng, SOUTH AFRICA; Candice Small, PhD, University of the Witwatersrand, Johannesburg, Gauteng 1709, SOUTH AFRICA

**Learning Overview:** After attending this presentation, attendees will understand how to use 3D-ID ([www.3d-id.org](http://www.3d-id.org)) to estimate ancestry and biological sex with coordinate data in forensic contexts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating an easy-to-use and freely available software that can be applied to estimate ancestry on a global scale.

Ancestry estimation is a critical component of the biological profile and in human identification. Traditionally, the estimation of ancestral origin is one of the most difficult parameters of the biological profile to assess, with accurate estimation hinging largely on the experience of the observer. Ancestry has traditionally been oversimplified as a trifecta of the continental populations of Asia, Europe, and Africa. However, this view ignores underlying microevolutionary forces such as gene flow, drift, and migrations that have shaped craniofacial form. 3D-ID addresses this limitation by grouping individuals based on known ethnohistorical origins.

3D-ID applies modern geometric morphometric analyses of three-dimensional landmark coordinates that provides more anatomical information than traditional linear caliper-derived methods. However, these newer modalities have yet to be widely applied in forensic anthropology and victim identification. One of the main humanitarian concerns is the identification of undocumented border crossers at the national and international level. This latest version of the software provides the tools to address this global humanitarian crisis of mass migration of individuals escaping wars, starvation, and displacement within and outside our borders. New reference samples have been added to the latest version of 3D-ID and include West African ( $n=93$ ), East African ( $n=36$ ), Nigerian ( $n=30$ ), Syrian ( $n=43$ ), and Colombian ( $n=71$ ) samples. The Nigerian sample was not grouped with the West African sample as it was found to vary significantly from the other samples included (e.g., Cameroon, Togo, Benin, Liberia, etc.). The sample from Colombia was also not grouped with the South American sample until the extent of variation among the groups (e.g., Chile, Peru) can be validated. The reference population included in the software totals 2,372 individuals from around the world. Another new feature added to the software to facilitate ease of use is that the practitioner can now digitize directly into the software without having to format the coordinate data.

The software was tested on a sample of Black South Africans ( $n=100$ ) from the Raymond A. Dart Collection of Human Skeletons that is not included in the software's reference population. The software had an 89% correct classification rate classifying 8 of the South African crania into "African" and 81 individuals into "African American" reference samples, which reflects the variation present on the African continent and underscores the need for addressing the migrant crisis on a global scale.

*This project was funded by a National Institute of Justice grant and a Forensic Technology Center of Excellence-RTI grant.*

#### Ancestry, Geometric Morphometrics, Software



### A40 A Reevaluation of the (hu)MANid Classification Software Using Virtually Collected Mandibulometer Measurements

Heather M. Garvin, PhD\*, Des Moines University, Des Moines, IA 50312-4198; Kelsey A. Carpenter, MS, Mercyhurst University, Erie, PA 16546; Gregory E. Berg, PhD, DPAA Identification Laboratory, Joint Base Pearl Harbor-Hickam, HI 96853-5530; Michael W. Kenyhercz, PhD, Department of Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI 96816

**Learning Overview:** After attending this presentation, attendees will understand how inaccurate mandibulometer measurements can impact sex and ancestry classifications and how the (hu)MANid classification software performs using validated virtually collected mandibulometer data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting an accurate method of collecting mandibulometer measurements from 3D models and a test of the (hu)MANid software on a virtual sample.

At a previous American Academy of Forensic Sciences (AAFS) meeting, Carpenter and Garvin presented a test of the (hu)MANid classification software that had been developed by Berg and Kenyhercz for sex and ancestry classification from mandibular metric and morphoscopic variables.<sup>1,2</sup> Carpenter and Garvin reported a strong bias toward the classification of White individuals into Black reference groups.<sup>1</sup> Following the AAFS meeting, discussions with the program developers and comparisons of group mean measurements with the (hu)MANid reference groups indicated that the tested mandibular length and maximum ramus height measurements were erroneous. The first goal of this study was thus to develop and test a method to accurately collect mandibulometer measurements (mandibular length, maximum ramus height, and mandibular/gonial angle) from 3D mandibular models. The second goal was to re-run the analyses on the same samples presented by Carpenter and Garvin to assess the impact of the erroneous mandibulometer measurements on group classification, as well as to test the (hu)MANid software program using accurate measurements.<sup>1</sup>

Geomagic Wrap® and Microsoft® 3D Builder were used to orient 3D mandibular models in lateral view as though they were resting on a flat surface. Screenshots of the lateral view (orthogonal projection) were extracted and opened in ImageJ for data collection. Horizontal and vertical guidelines were placed so a mandibular angle consistent with that measured with a mandibulometer could be measured and drawn, with subsequent mandibular lengths and ramus heights collected using the guidelines and lengths from the drawn angle. A sample of 89 mandibles, in which both 3D scans and mandibulometer measurements were available, was used to test the accuracy of the virtual data collection method. Relative Technical Error of Measurements (rTEM) were within acceptable levels, with 1.69% for angle measurements, 1.65% for length measurements, and 3.41% for ramus height measurements. The higher ramus height errors may be related to difficulties in observing the superior extent of the condyles in the 3D scans given that the mandibles had been originally scanned in articulation with the associated cranium. Intra-observer analyses performed on 20 mandibular scans returned rTEM values of 0.61% for angle, 0.75% for length, and 1.20% for ramus measurements.

When the revised mandibular length and ramus height measurements and the additional mandibular angle measurements were utilized to re-run the Carpenter and Garvin analyses on the same sample of 230 United States White and Black mandibles, classifications improved.<sup>1</sup> Using Linear Discriminant Analysis (LDA), correct sex classifications up to 83.12% were obtained for the pooled sample, and the previously reported sex biases were not observed. Correct group classifications (sex+ancestry) were similar to those reported by Berg and Kenyhercz.<sup>2</sup> Group classifications as high as 78.7% were obtained when the sample was run against only White and Black reference groups, although some ancestry bias was observed with higher classifications for White males and females (WM=78.68%, WF=70.68%, BM=56.14%, BF=29.62%). Mixture Discriminant Analysis (MDA) was also used and typically outperformed the LDA in total correct classification, but the upward bias in classification of Whites remained. The lower classifications of the Black females may represent smaller reference group sample sizes and may improve as additional samples are added to (hu)MANid. In addition to providing a test of the (hu)MANid program, this study highlights the diligence required when overcoming research challenges, as well as the need to transform traditional methods as data and methods advance with increased accessibility to 3D data.

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1. Carpenter K.A., Garvin H.M. A Test of the (hu)MANid Classification Software on a Sample of United States White and Black Mandibles. *Proceedings of the American Academy of Forensic Sciences, 70<sup>th</sup> Annual Scientific Meeting, Seattle, WA. 2018. A56.*
2. Berg G.E., Kenyhercz M.W. Introducing Human Mandible Identification [(hu)MANid]: A Free, Web-Based GUI to Classify Human Mandibles. *J Forensic Sci.* 2017; 62:1592-1598.

#### Mandible, Sex Estimation, Ancestry Estimation



## A41 Craniometric Variation and Sexual Dimorphism in a Chilean Population

Rachel M. Kreher, BS\*, University of Indianapolis Biology Department, Indianapolis, IN 46227; Krista E. Latham, PhD, University of Indianapolis, Indianapolis, IN 46227; Stephen P. Nawrocki, PhD, University of Indianapolis, Indianapolis, IN 46227-3697

**Learning Overview:** After attending this presentation, attendees will better understand the strengths and weaknesses of using FORDISC® to analyze Chilean crania. The learning objectives include better understanding of human biological variation in a Latin American subgroup and how the accuracy of sex determination can be improved in forensic contexts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating that not all Hispanic populations in the New World are skeletally identical, so existing analytical methods must be carefully assessed prior to application.

This research tests the following null hypotheses: (1) there are no significant differences between Chilean males and females in standard cranial measurements; (2) these measurements are not affected by age at death; and (3) Chileans do not differ from other Hispanic reference samples in FORDISC®v.3.1.

In the United States, many sex and ancestry determination methods used by forensic anthropologists are based on White and Black reference populations, but these methods may not work well on other populations. “Hispanic” individuals of Spanish (Iberian) and Latin American descent are especially underrepresented. In Chile, accurate sex determination is crucial in forensic investigations as the country continues to address prior genocides and experiences a recent influx of migrants from surrounding countries. This research examines whether currently available methods can accurately determine sex of Chilean crania, specifically investigating the application of discriminant analysis via FORDISC® to determine whether the program’s Hispanic reference sample adequately represents modern Chileans.

A total of 27 standard cranial measurements were taken for 250 specimens from the Cementerio General Collection located at the Universidad de Chile and the Servicio Médico Legal in Santiago, Chile. The modern sample includes known adult males and females aged ~16–80+ years who died in the 1950s–1990s. All measurements were first analyzed with Analysis of Covariance (ANCOVA) to determine which vary significantly by sex and/or age. Subgroup means were generated for each sex for comparison to the reference samples. The Bonferroni correction was applied to reduce the probability of Type I (false positive) errors in the ANCOVAs. Each specimen was then entered into FORDISC® and subjected to a 9-subgroup discriminant analysis using the White, Black, American Indian, Hispanic, and Guatemalan reference samples. A stepwise selection of 15 variables was employed to ensure that a sufficient number of measurements were included without over-fitting the model. Primary ancestry/sex classification was recorded in addition to relevant distance values and classification probabilities. For the purposes of this study, “Hispanic” and “Guatemalan” classifications were both considered to be “correct.”

Of the 27 cranial measurements, 20 differed significantly between the sexes, with males being larger in all cases. Age at death was significant for only four measurements, but, in each case, the effects of age were much less important than the effects of sex. Therefore, discriminant equations for determining sex can reasonably disregard the age of the decedent.

Only 32% of the Chileans were classified correctly by FORDISC® into their proper ancestry/sex subgroup; 39% of Chilean females were classified as Hispanic females, while 26% of Chilean males were classified as Hispanic or Guatemalan males. The most common ancestry groups assigned to Chilean females were Hispanic (41%), White (29%), and Black (25%); for Chilean males, Hispanic/Guatemalan (36%), White (26%), and Black (24%) were the most common assignments. Ignoring ancestry, 97% of Chilean females were classified as female, while only 55% of Chilean males were classified as male. This disparity suggests that the entire Chilean sample is systematically smaller than FORDISC’s® Hispanic reference sample. Comparisons of the Chilean means by sex with those of Hispanics in FORDISC® indicate that Chilean females are smaller than their FORDISC® counterparts in 24 measurements and Chilean males are smaller than their FORDISC® counterparts in 22 measurements. These differences explain the high and low classification rates for Chilean females and males, respectively: because *all* Chileans are smaller than the reference samples, they are disproportionately classified as female.

In sum, despite their overall smaller size, modern Chileans display enough sexual dimorphism that tailored discriminant equations should work well for forensic anthropologists. These findings reinforce the need for a population-specific understanding of body size and sexual dimorphism prior to applying sex and ancestry determination methods, especially given the wide range of geographical and biological variation collected under the umbrella designation of “Hispanic.”

### FORDISC®, Chilean Craniometrics, Sex Determination



### A42 The Application of the Optimized Summed Scored Attributes (OSSA) Method to Sex Estimation in Whites and Blacks

Summer M. Mizell\*, Washburn University, Topeka, KS 66614; Holly Long\*, Stilwell, KS 66085; Alexandra R. Klales, PhD, Washburn University, Topeka, KS 66621

**Learning Overview:** After attending this presentation, attendees will understand the practicality of the OSSA method for sex estimation in unidentified individuals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by validating a new method of sex estimation and demonstrating how this method can be incorporated into the biological profile aspect of casework.

Recently, Tallman and Go applied the OSSA method, originally developed for ancestry estimation, to the five cranial traits of Walker in order to estimate sex.<sup>1,2</sup> While possessing great potential as a new sex estimation method, this approach has only been tested in Asian samples, which had a high sex bias (>24.5%) despite high classification accuracy (83.7% calibration; 81.9% validation). This research tests the applicability of the OSSA sex estimation method in United States Black and White populations. A random sex/ancestry balanced sample of 700 individuals from the MorphoPASSE Database Databank (MDD) was utilized to calibrate the OSSA method dichotomized scores and sex classification sectioning points. Everyone in the MDD had been previously scored for the five Walker traits by an experienced observer from the Terry Anatomical, William Bass Forensic Skeletal, and the Hamann-Todd Human Osteological Collections.<sup>1</sup> A second random validation sample of 200 individuals from the MDD was used to test the validity of the OSSA method.

First, trait frequencies of the nuchal crest, glabella, mastoid, supraorbital margin, and mental eminence were calculated in the calibration sample by sex/ancestry group for each score (from one to five). The trait scores were then dichotomized based on the frequency distributions and score mean, median, and modes. Trait scores that were more frequent in females were dichotomized to a zero, while trait scores more frequent in males were dichotomized to one. The scores for all five cranial traits were summed to obtain a combined OSSA score from zero to five. From these totals, the sectioning point for sex classification was determined based on classification accuracy rates in the calibration sample. Finally, the validity of the OSSA sex method was tested in the holdout sample.

In Blacks, the nuchal crest, glabella, mastoid, and orbital scores of 1–2 were dichotomized to an OSSA score of 0, while scores 3–5 were dichotomized to an OSSA score of 1. For the mental eminence, scores 1–3 were designated as a 0 and scores of 4–5 were designated as a 1. A summed OSSA score of  $\leq 2$  classified as female, while  $\geq 3$  classified as male. Classification accuracy in the calibration sample was 77.4% with 15.4% sex bias in favor of males, while validation accuracy in the holdout sample was 78.0% with 8.0% sex bias in favor of males. In Whites, the nuchal crest, glabella, mastoid, and mental eminence scores of 1–2 were dichotomized to an OSSA score 0, while scores 3–5 were dichotomized to an OSSA score 1. For the orbital margin, scores 1–3 were designated as a 0 and scores 4–5 were designated as a 1. A summed OSSA score of  $\leq 3$  classified as female, while  $\geq 4$  classified as male. Classification accuracy in the calibration sample was 77.1% with 20.6% sex bias in favor of females, while validation accuracy in the holdout sample was 70% with a 56% sex bias in favor of females.

Walker's method using these five traits and logistic regression produced 87.9% correct classification and 0.1% sex bias in his sample and logistic regression analysis in this sample produced 83.0% accuracy and 0.2% sex bias, which suggests that employing his trait scores in logistic regression far outperforms the OSSA sex estimation method and had considerably less sex bias<sup>2</sup>. One option to improve classification accuracy and the utility of OSSA for sex would be the inclusion of an indeterminate category as proposed by Tallman and Go, and then only utilize the method for individuals that do not have an indeterminate summed OSSA score.<sup>1</sup> In conclusion, this research suggests that the OSSA method can be applied to sex estimation in United States forensic casework; however, the results are less accurate and have a higher sex bias than using the original Walker traits in regression analyses.<sup>2</sup>

#### Reference(s):

1. Tallman, S.D., and Go, M.C. (2018). Application of the Optimized Summed Scored Attributes Method to Sex Estimation in Asian Crania. *Journal of Forensic Sciences*, 63(3), 809-814.
2. Walker, P.L. (2008). Sexing Skulls Using Discriminant Function Analysis of Virtually Assessed Traits. *American Journal of Physical Anthropology*, 136, 39-50.

#### Walker Traits, Sex Estimation, OSSA Method





## A43 Worldwide Population Variation in Skull Sexual Dimorphism

Alexandra R. Klales, PhD\*, Washburn University, Topeka, KS 66621; Stephanie J. Cole, MS, University of Nevada, Reno, Reno, NV 89557; Kyra E. Stull, PhD, University of Nevada, Reno, Reno, NV 89557; Heather M. Garvin, PhD, Des Moines University, Des Moines, IA 50312-4198; Matthew C. Go, MA, University of Illinois at Urbana-Champaign, Urbana, IL 61801; Antinea Menéndez Garmendia, MS, Universidad Nacional Autónoma de México, Mexico City 04510, MEXICO; Mackenzie Walls, Forensic Anthropology Program, Topeka, KS 66621; Gabriela Sánchez-Mejorada, PhD, Universidad Nacional Autónoma de México, Ciudad de México 04510, MEXICO

**Learning Overview:** After attending this presentation, attendees will understand how five popular morphological traits of the skull utilized for sex estimation vary within and between populations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a validation of the Walker method using multiple populations and providing new recalibrated population-specific equations, as well as a global equation that can be utilized when ancestry is unknown or inconclusive.<sup>1</sup>

Due to varying levels of sexual dimorphism between populations, sex is typically estimated after general age (i.e., adult vs. subadult) and ancestry estimation. The Walker method remains the most popular morphological method using the skull; however, validations of this method using different populations have varied.<sup>1</sup> To better examine worldwide variation in these morphological skull traits, a large global sample of 2,285 skulls were scored by this study using descriptions and illustrations of the nuchal crest, mastoid process, glabella, mental eminence, and supra-orbital margin provided by Walker.<sup>1</sup> Analyses were run to assess geographic population (e.g., United States, Nubian, South African, Philippines, and Mexico) differences. A Fisher's exact test with Monte Carlo simulation was used to test for sexual dimorphism in trait score frequencies between females and males for each trait within each geographic region. To further examine differences between geographic regions, a Kruskal-Wallis test was used and, if rejected, a Dunn's test using a Holm's stepwise adjustment was employed to determine which groups were significantly different from one another. Next, the external validity of Walker's method was tested by entering scores from each skull into the six logistic discriminant function equations provided by Walker.<sup>1</sup> Finally, recalibrated logistic regression equations were calculated for each geographic population and for the global (i.e., all groups combined) sample. Classification accuracies obtained using the recalibrated equations were compared to the validation results, as well as the classification accuracies generated using the global equation, to determine if population-specific equations were necessary.

Significant differences between males and females within groups were found for all traits ( $p$ -values $\leq 0.016$ ), except for the nuchal crest in Nubians ( $p=0.06$ ) and mental eminence in South African Whites ( $p=0.80$ ), indicating sexual dimorphism is present in nearly all traits across groups. Significant differences ( $p < 0.001$ ) for both males and females for all five traits were found using the Kruskal-Wallis test and between pairwise comparisons of groups using the Dunn's test, which most notably indicated significant differences in the expression of glabella in United States White females and the mastoid process in Mexican males when compared to all other groups. Validation classification accuracies using Walker's equations ranged from 65.8% (United States Blacks, equation 5) to 88.2% (Philippines, equation 2), depending on the equation utilized and population examined.<sup>1</sup> Overall, equation 1, utilizing glabella, mastoid, and mental eminence, performed the best (77.3%–85.7%); however, sex bias was as high as 42.1%. As expected, classification accuracy typically improved with recalibration in nearly every group. Improved accuracy ranged between 0.1% (United States Whites, equation 6) and 16% (South African Whites, equation 4). Accuracy rates using a recalibrated global equation (i.e., all geographic regions) resulted in accuracy rates ranging from 74.1% (equation 5 using the orbit and mental eminence) to 81.8% (equation 1 using glabella, mastoid, and mental eminence). Some groups performed better in the global equation than the group-specific equations, most notably so with the Mexican sample. However, this increased accuracy often came at the expense of a higher sex bias. In conclusion, this work highlights the complexity of sexual dimorphism in different populations and the need for population-specific equations for these skull traits in the Walker method; however, when ancestry (or geographic region) is unknown or inconclusive, a global equation can be utilized with acceptable accuracy rates.<sup>1</sup>

Portions of this research were funded by National Institute of Justice (NIJ) grants. Opinions, points of view expressed in this research, and products discussed represent a consensus of the authors and do not necessarily represent the official position, policies, or endorsement of the United States Department of Justice (DOJ) or NIJ. Data collection for the Filipino sample was also funded by the Social Sciences and Humanities Research Council of Canada.

### Reference(s):

<sup>1</sup>. Walker P.L. Sexing Skulls Using Discriminant Function Analysis of Visually Assessed Traits. *Am J Phys Anthropol.* 2008; 136:39-50.

### Walker Method, Sex Estimation, Population Variation



## A44 Cognitive Bias and the Order of Examination on Skeletal Remains

*Marion E. Davidson\*, Metuchen, NJ 08840; Carolyn Rando, PhD, University College London, London WC1H 0PY, UNITED KINGDOM; Sherry Nakhaeizadeh, University College London (UCL), London WC1H 9EZ, UNITED KINGDOM*

**Learning Overview:** After attending this presentation, attendees will understand how the order of analysis utilized in a morphological examination of human skeletal remains can potentially influence the judgements and decisions of forensic anthropologists.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating that cognitive bias can result from initial exposure to one aspect of skeletal remains and affect the final sex determination. This presentation will also discuss the importance of empirical research in cognitive bias to develop frameworks that incorporate an understanding of when such biases may influence the interpretation of evidence.

The National Research Council in the United States identified potential cognitive bias by experts as a complication facing the forensic science community and noted that fields utilizing subjective methods with human interpretation are particularly susceptible. Studies have demonstrated that cognitive bias because of exposure to extraneous contextual information exists in various forensic fields and can influence the judgement of examiners. Despite these results, there continues to be a shortage of empirical research into the extent of cognitive bias within the various stages of the forensic process, such as data collection, analysis, and interpretation. Although research into cognitive bias in forensic anthropology remains low, several studies have demonstrated that the determination of the biological profile and the analysis of trauma can be influenced by extraneous information or initial visual exposure to context. This study was designed to assess the potential of cognitive biases resulting from the initial visual exposure to one aspect of skeletal remains for sex determination and its effects on subsequent aspects as well as the final sex determination.

A pilot study was developed to assess the potential of cognitive bias in the frequently employed morphological sex determination methods as a result of the order of examination. A total number of 30 participants was divided into two groups and asked to assess one complete skeleton for both sex and age, employing only morphological methods of the skull and pelvis. Age at death was only included as a variable to provide a more realistic evaluation for the participants. Group A examined the skull, followed by the pelvis, while Group B examined the pelvis, followed by the skull. As cognitive bias occurs more predominantly in ambiguous cases, the skeleton utilized in this study displayed some amount of ambiguity, with the pelvis exhibiting strongly male features and the skull exhibiting indeterminate to female features. To minimize any potential influence on the decision-making process, participants in this study were not informed of the true nature of the experiment.

The results of the pilot study determined that the order in which the skeletal remains were examined influenced the participant's subsequent analyses and final sex determination. When analyzed in three categories (female/probably female, indeterminate, and probably male/male), the results indicated a difference in the sex determination of the two groups, depending on the order of the analyses. For example, Group A, after first examining the female/indeterminate skull, assessed the pelvis to be female at a rate of 53.30%, while Group B, examining the pelvis first, assessed it to be female at a rate of only 6.67%. Similar results were found in the final sex determination, with Group A assessing the skeletal remains to be female at 60.00%, while Group B assessed the skeletal remains to be female at only 13.30%. This presentation will present the results of this research and discuss the susceptibility of these methods to cognitive bias and the importance of studying these biases to mitigate the effects and improve the field of forensic anthropology.

### Forensic Anthropology, Cognitive Bias, Sex Determination



### A45 The Impact of Bone Density and Bone Thickness on Gunshot Trauma to the Skull

Elizabeth A. Evangelou, MA\*, Binghamton University, Binghamton, NY 13905; Tessa Somogyi, MA\*, Binghamton University, Binghamton, NY 13902; Alexis M. LaGoy\*, Baldwinsville, NY 13027; Kevin E. Sheridan, PhD\*, Binghamton University, Binghamton, NY 13902; Elizabeth A. DiGangi, PhD\*, Department of Anthropology, Binghamton, NY 13902

**Learning Overview:** After attending this presentation, attendees will gain awareness of: (1) how bone mineral density and bone mineral content affect the propagation of radiating fractures and the overall fragmentation of the skull following a gunshot wound; and (2) how bone thickness affects the size and morphology of entrance and exit wounds.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating and discussing how bone density and bone thickness affect the formation of radiating fractures and the size/shape of entrance and exit wounds.

A greater understanding of the tissue density in the region of the gunshot may allow researchers greater estimation powers when estimating bullet caliber.<sup>1</sup> Bone mineral density and mineral content represent an understudied and potentially fruitful avenue of gunshot research. Here, measurements of bone mineral density are utilized to assess the density of tissue at the point of gunshot impact and the effect that differing densities have on the morphology of entrance and exit wounds, as well as on radiating fractures.

Forty-five adult human heads from individuals donated to an anatomical tissue supply company were obtained for traumatic analysis. A specially designed shooting stand was constructed to hold each head at a height matching that of an average adult male. Individuals were shot once in either the frontal bone (superior to glabella), temporal, or parietal bone (in the approximate region of euryon) with a Smith and Wesson revolver with a 1<sup>7</sup>/<sub>8</sub>-inch barrel loaded with .38 Special bullets. Bullets used were full metal jacket and jacketed hollow points, and these were chosen at random and equally distributed.

The heads were processed using standard maceration techniques prior to being scanned for bone mineral density and bone mineral content using Dual Energy X-ray Absorptiometry (DEXA). Bone thickness was measured within 5mm of the entrance and exit wounds using spreading calipers. The radiating fractures were measured using a Scale Master digital plan measure. Fragmentation of the skull was assessed by sorting individuals into categories based on degree of fragmentation: 1=no fragmentation; 2=partial fragmentation, but wound shape still evident; 3=partial fragmentation, wound shape obscured; and 4=complete fragmentation. Shape of the entrance and exit wounds was assessed using the circularity function in the image processing program ImageJ.

A Pearson product-moment correlation analysis was performed to assess the relationship between bone mineral density, bone mineral content, and bone thickness (entrance and exit) and the size/morphology of entrance and exit wounds, the extent of radiating fracture formation, and the degree of fragmentation of the skull (significance set at  $p=.05$ ). Preliminary results indicate there is no significant correlation between bone mineral density and bone mineral content and the size of entrance and exit wounds. There are moderate negative correlations between bone mineral density and bone mineral content and the total length of the exit wound radiating fractures ( $r=-.492$ ,  $p=.013$ ;  $r=-.505$ ,  $p=.010$ ), the maximum length of the exit wound radiating fractures ( $r=-.562$ ,  $p=.003$ ;  $r=-.543$ ,  $p=.005$ ), and the degree of fragmentation ( $r=-.427$ ,  $p=.010$ ;  $r=-.421$ ,  $p=.021$ ). Entrance wound thickness had a slight positive correlation with entrance wound perimeter ( $r=.336$ ,  $p=.028$ ) and a slight negative correlation with entrance wound circularity ( $r=-.325$ ,  $p=.033$ ). Exit wound thickness had no significant correlations, nor did exit wound size or shape.

These results have implications for future gunshot wound analysis, as they suggest that bone mineral density and bone mineral content have more of an effect on the propagation of radiating fractures and the degree of fragmentation of the skull than they do on entrance and exit wound size and shape. These data also suggest that bone thickness near the entrance wound affects entrance wound size and shape. Future research will focus on how this knowledge of the effects of bone density and bone thickness can help distinguish between bullet types and perhaps also bullet calibers.

This project was supported by an award from the National Institute of Justice.

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#### Gunshot Analysis, Bone Mineral Density, Radiating Fracture Analysis



### A46 A Comparison of Bullet Construction to the Area and Perimeter of Gunshot Entrance and Exit Wounds

Tessa Somogyi, MA\*, Binghamton University, Binghamton, NY 13902; Elizabeth A. Evangelou, MA\*, Binghamton University, Binghamton, NY 13905; Alexis M. LaGoy\*, Baldwinsville, NY 13027; Kevin E. Sheridan, PhD\*, Binghamton University, Binghamton, NY 13902; Elizabeth A. DiGangi, PhD\*, Department of Anthropology, Binghamton, NY 13902

**Learning Overview:** After attending this presentation, attendees will gain awareness of: (1) how the sizes (area and perimeter) of entry and exit wounds on the human skull are affected by the bullet construction (full metal jacket vs. jacketed hollow point); and (2) the implications of the relationship between the size of entrance and exit wounds to bullet construction and how this can be utilized within a medicolegal setting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating and discussing how bullet construction affects the size of entry and exit wounds on the human skull.

The interpretation of gunshot wounds is a key element in skeletal trauma analysis. Many interpretations of gunshot and other types of skeletal trauma have been based on observations made during autopsies or observations from osteological materials recovered from archaeological contexts.<sup>1</sup> There has been a lack of controlled gunshot trauma experiments to add to these data from autopsy and archaeology. Controlled experiments are necessary to allow for detailed observations and interpretations of the interactions between intrinsic and extrinsic factors, both of which affect patterns of skeletal trauma.<sup>1</sup> Extrinsic factors include a wide variety of factors such as the type of firearm, bullet caliber, and bullet construction. Previous research has been able to reveal some patterns, allowing the prediction of bullet caliber or other bullet characteristics under controlled conditions, but much more research is needed in this area to allow for more complete interpretations. A controlled experimental approach is the best way to meet this need. Therefore, this project experimentally tested the effects of two different bullet constructions (full metal jacket and jacketed hollow point) on the size (area and perimeter) of entrance and exit wounds to the cranium. These bullet types were chosen because they are designed to do different things to human tissue (i.e., exit the body vs. not exit) and tend to be used in different contexts.

Forty-five human heads from donated individuals were sourced from an anatomical tissue supply company, specifically for the purpose of trauma research. Prior to shooting, each head was placed on a specialized stand set at the height of an average adult male. Each head was shot once either through the frontal or through the temporal or parietal bone using a Smith and Wesson® model 438 J-frame revolver with a 1<sup>7</sup>/<sub>8</sub>-inch barrel loaded with .38 Special bullets. Bullet construction (jacketed hollow point vs. full metal jacket) was distributed randomly throughout the sample with equal numbers of each bullet construction used. Following the shooting, heads were autopsied and processed using standard maceration techniques.

The area and perimeter of the entrance and exit wounds were measured virtually using ImageJ, a freely available image processing program. To test the interaction between bullet construction and the area and perimeter of entrance and exit wounds, a series of independent sample *t*-tests were conducted with significance set at  $p=.05$ . Earlier research has indicated there is a relationship between entrance and exit wound sizes, with exit wounds almost always larger than their associated entrance wounds. The relationship between entrance and exit wound relative size (exit wound size divided by entrance wound size) and bullet construction was also tested here.

Preliminary data analysis revealed that there is a significant difference between bullet construction for the area and perimeter of the entrance wounds, where jacketed hollow points produce entrance wounds with larger areas and perimeters than those produced by full metal jackets ( $p=.001$  for both). Preliminary results also reveal there is no significant difference between the two bullet types in the area and perimeter of exit wounds. The preliminary results analyzing entrance and exit wound relative size indicate that the exit wounds are consistently larger than their associated entrance wounds; however, there is no statistically significant relationship between the ratio of the two wound sizes and bullet construction. This has implications for gunshot wound fracture analysis in that these results reveal bullet construction can significantly affect entrance wound morphology. Future research will include analysis of fracture pattern differences by bullet type and the interaction between bullet type and entrance wound location.

*This project was supported by an award from the National Institute of Justice.*

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#### Forensic Anthropology, Gunshot Trauma, Trauma Analysis



### A47 Differential Mass Loss in Bullets From Frontal and Temporal/Parietal Bone Impacts

*Kevin E. Sheridan, PhD\**, Binghamton University, Binghamton, NY 13902; *Donald Poon, BA*, Department of Anthropology, Binghamton, NY 13902; *Jessica E. Sanger, BA*, Binghamton, NY 13903; *Elizabeth A. Evangelou, MA*, Binghamton University, Binghamton, NY 13905; *Tessa Somogyi, MA*, Binghamton University, Binghamton, NY 13902; *Elizabeth A. DiGangi, PhD*, Department of Anthropology, Binghamton, NY 13902

**Learning Overview:** After attending this presentation, attendees will understand the potential for using mass loss in bullets as an indicator of which human skeletal element caused said deformation. Attendees will gain knowledge of the various factors contributing to bullet mass loss, including skeletal element, bullet type, distance, and velocity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through the development of an additional tool for reconstructing crime scenes. This information will be particularly useful in understanding cases in which bullets are recovered in the absence of human remains.

The effect of ballistic impacts upon bone tissue is a frequent topic of research within forensic anthropology. However, there is a dearth of research regarding the effect of ballistic trauma on bullet condition. There is limited systematic research dealing with the effect of differential bone density on bullet deformation, particularly concerning human skeletal tissue.

This study examines the differential deformation of .38 Special bullets fired into donated human heads as part of an experimental study on the effects of bullet type on head trauma. Ammunition utilized during the study included both Full Metal Jacket (FMJ) and Hollow Point Type (JHP) bullets, both of which were 130 grains in weight. Bullets were fired into the frontal bone and right temporal/parietal area. A total of 31 bullets were used for this analysis. The bullets were nearly evenly distributed between FMJ ( $n=16$ ) and JHP ( $n=15$ ). Bullets shot to the frontal bone numbered 18, while bullets shot to the temporal bone numbered 13.

Damage to each bullet was quantified as mass loss measured in grains. Bullets were weighed following recovery, and mass loss was measured as 130 grains minus the weight of each recovered bullet. The results were analyzed via univariate Analysis of Covariance (ANCOVA) through SPSS software.

Analysis of the mass loss exhibited substantially greater mass loss from bullets fired into the frontal bone than in those fired into the temporal. In the full sample, the mean average loss of mass for hits to the frontal bone was 10.448 grains, while hits to the temporal bone averaged 1.032 grains. This represents a mean average loss of approximately 8% for frontal bone hits versus 0.8% for the parietal/temporal. However, the standard deviation for frontal hits was substantially larger (15.588 grains) than the standard deviation for temporal hits (1.623 grains). Mass loss for bullets fired into the frontal bone ranged from 0–51.76 grains. By contrast, mass loss for bullets fired into the temporal bone ranged from 0–6.23 grains.

The relationship between loss of bullet mass, impacted bone, and bullet type were analyzed via univariate ANCOVA. The results for the full sample exhibited a significant relationship between mass lost and target bone on the skull ( $p=0.039$ ). By contrast, bullet type (FMJ vs. JHP) exhibited no significant relationship with mass lost ( $p=0.429$ ). The combined effect of target and bullet type exhibited no significant relationship ( $p=0.668$ ).

The differential degrees of bullet mass loss between skeletal elements observed in this study suggest that this figure may be used as a metric for associating fired bullets with damage to skull elements of differing thickness. At lower levels of mass loss ( $>1.3$  grains), distinction between frontal and temporal damage would not be possible, since the ranges of mass loss overlap at these levels. At greater levels of mass loss, bullets are more likely to be associated with hits to the frontal bone. In general, this supports the hypothesis that thickness has a significant influence on the degree of damage to bullets striking bone.

This study is limited to the effect of a single handgun caliber. Nevertheless, the results of this study will contribute to the available methodologies for forensic scientists for reconstructing crime scenes, particularly those involving situations in which bullets are recovered without associated human remains.

*This project was supported by an award from the National Institute of Justice.*

### Ballistic Trauma, Firearms, Forensic Anthropology



### A48 Scanning Electron Microscopes With Energy Dispersive X-Ray Spectroscopy (SEM/EDX) Analysis of Gunshot Residue (GSR) on Pig Bone

Anthony B. Falsetti, PhD\*, George Mason University, Fairfax, VA 22030; Natalie R. Langley, PhD, Mayo Clinic School of Medicine, Scottsdale, AZ 85259; Blake D. Ehlers, Arizona State University, Chandler, AZ 85225; Bethany M. Pittman, Arizona State University, Glendale, AZ 85304; AmberCherie Lasala, Arizona State University, Glendale, AZ 85306-4908; Kimber G. Cheek, BS, University of Tennessee, Knoxville, Fredericksburg, VA 22408; Derek A. Boyd, MA, University of Tennessee, Knoxville, Knoxville, TN 37996

**Learning Overview:** After attending this presentation, attendees will understand how SEM/EDX may be used to detect and confirm evidence of particulate matter associated with GSR (powder and primer) on pig cranial bone.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a means for trauma on bone considered to be from a firearm to be confirmed through SEM/EDX instead of relying solely on visual assessment.

Anthropologists examine decomposed or skeletonized remains where trauma is interpreted through evaluating patterns of altered bone. Distinguishing between projectile and blunt trauma is complicated by postmortem processes that result in fragmentation or loss of surface features. The presence of projectile fragments in bone or surrounding tissues has long been known to be associated with projectile and gunshot events.<sup>1,2</sup> However, such fragments are not always present, and their absence cannot exclude a gunshot event. The presence of other evidence associated with a firearm discharge may be another indicator that a gunshot event has occurred.

When a firearm is discharged, the primer mix containing lead styphnate, antimony sulfide, and barium nitrate is crushed by a firing pin. The gas that propels the bullet is partly comprised of derivatives of the primer mix, including lead (Pb), barium (Ba), and antimony (Sb), and condenses into droplets that can leave a residue (GSR) on nearby surfaces. Primer residue is utilized in forensic analyses to determine proximity to a firearm, as the residue is commonly deposited on nearby objects, such as the shooter's hands and/or clothing.<sup>3</sup> A finding of GSR primer on bone would justifiably be indicative of a gunshot event within close proximity of the bone.

SEM/EDX have been used to detect GSR on multiple surfaces, including bone.<sup>4,5</sup> SEM/EDX is an analytical method that can determine the elemental composition of single particles. This study prepared samples of *Sus scrofa* for SEM/EDX analysis of gunshot injuries created from three distances (direct contact, 6", and 12"). After wounding, the specimens underwent either outdoor decomposition or warm water maceration. The six heads were sectioned along the mid-sagittal plane, resulting in a total of 12 specimens.

Tescan Vega 3 software was used to control the SEM, while the AZtec software was used to control the EDX. Elemental analysis was performed by the EDX software AZtec. AZtec provides a graph of X-ray values read from the sample, which is then compared to the known X-ray values table, giving the exact element present.

For the 12 specimens, evidence of gunshot powder and primer were discovered, though as distance increases, the number and type of particle detected decreases. For example, both primer and power (Pb, Ba, and Sb) elements were detected from wound tracts from contact samples as well as from surrounding fragments. Specimens that were left to decompose and those that were water macerated provided mixed results. Most samples provided elements that were expected from GSR, but a surprisingly few gave unexpected elements. Some of the unexpected elements, such as iron (Fe) and aluminum (Al), can be found in soil, but others, such as silicon (Si), do not provide any provenance. A limitation of this process is that every sample must be completely dry as the SEM/EDX operates under a vacuum.

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#### SEM/EDX, Gunshot Residue, Bone



### **A49 Thermogravimetric Analysis (TGA) of Juvenile Pig Rib Compositional Changes**

*Luisa Marinho, MSc\*, Simon Fraser University, Burnaby, BC V5A1S6, CANADA; Dev Sharma, PhD, Simon Fraser University - Department of Chemistry, Burnaby, BC V5A1S6, CANADA; Carolyn J. Sparrey, PhD, Simon Fraser University, Surrey, BC V3T0A3, CANADA; Stephen N. Robinovitch, PhD, Simon Fraser University, Burnaby, BC V5A1S6, CANADA; Alison Galloway, PhD, University of California, Santa Cruz, CA 95064; Hugo Cardoso, PhD, Simon Fraser University, Burnaby, BC V5A 1S6, CANADA*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### A50 The Effects of Chemotherapy and/or Radiotherapy Treatment on Decomposition

Shelby Garza, BS\*, Texas State University, San Marcos, TX 78666; Daniel J. Wescott, PhD, Texas State University, San Marcos, TX 78666-4684

**Learning Overview:** After attending this presentation, attendees will better understand how chemotherapy and/or radiotherapy affect the rate of decomposition in human remains in an outdoor setting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding to the ongoing research being conducted involving variables that can affect human decomposition.

Understanding how chemotherapy and/or radiotherapy affects the rate of decay is important in a forensic setting. Individuals who died while undergoing chemotherapy or radiotherapy have the potential to decompose at a slower rate, complicating the estimation of the postmortem interval in criminal and civil cases. There are also instances in which taphonomic research facilities receive donated individuals that either died while undergoing chemotherapy or had previously stopped chemotherapy treatment.

Chemotherapy and radiotherapy are both common practices used to fight cancer in individuals. While chemotherapy uses chemical substances to treat the disease, radiotherapy is a more localized attempt to fight the disease with forms of radiation. While the chemicals used during chemotherapy have the potential to slow the rate of decay, the half-life of the drugs used in chemotherapy can be as little as six hours.<sup>1</sup> The purpose of this study is to examine the difference in decomposition between individuals who had undergone chemotherapy or radiation at or near the time of death and individuals who had never undergone chemotherapy or radiation. Currently, there have been no studies that have used whole body donations in an outdoor setting to examine differences in decomposition between individuals who had undergone chemotherapy or radiation at or near the time of death and individuals who had never undergone chemotherapy or radiation.

For this study, a total of ten human remains that had been left to decompose in an outdoor setting at the Forensic Anthropology Center at Texas State were used. Five of the donations had undergone chemotherapy or radiation at or near the time of death and were pair matched to remains that had never had chemotherapy treatment using body size and season of placement. All remains were placed unclothed and on the ground surface under a wire cage to prevent scavenging. A Total Body Score (TBS) was calculated for each subject at approximately 100, 300, and 500 Accumulated Degree Days (ADD).<sup>2</sup>

An F-Test was conducted to explore the homogeneity of variance between groups. Results for 100, 300, and 500 ADD indicated there was a statistical difference in variance of TBS between groups ( $F(1, 18)=0.314, p=0.144$ ;  $F(1, 18)=0.358, p=0.172$ ;  $F(1, 18)=0.685, p=0.362$ ). A two-tailed *t*-test assuming unequal variance was run to test for significance in 100, 300, and 500 ADD. The P value states that there is no significant difference between groups at 100, 300, and 500 ADD ( $p=0.596$ ;  $p=0.285$ ;  $p=0.346$ ).

This research fails to reject the null hypothesis, showing there is no significant difference in rate of decomposition between individuals that had undergone chemotherapy or radiation at or near the time of death and individuals that had never undergone chemotherapy or radiation.

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#### Decomposition, Chemotherapy, Total Body Score





### A51 Time to Skeletonization (TTS): *Dermestes maculatus* De Geer Skin/Hide Beetles and Skeletonization on *Python Bivittatus* Kuhl Burmese Python Remains

Amy Pham, BA\*, Fort Myers, FL 33967; Shannon L. Dery\*, Fort Myers, FL 33967-2503; Heather A. Walsh-Haney, PhD, Florida Gulf Coast University, Fort Myers, FL 33965-6565; Jason H. Byrd, PhD, University of Florida, Gainesville, FL 32608; Ian Bartoszek, Conservancy of Southwest Florida, Naples, FL 34102

**Learning Overview:** After attending this presentation, attendees will understand the effect of using a *D. maculatus* (skin/hide beetles) to create a TTS baseline for flesh removal in a controlled laboratory setting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing the process of rendering forensic cases for subsequent forensic anthropological analysis and to set a baseline for *D. maculatus* TTS in a controlled environment.

Cleaning techniques of skeletal remains conducted through maceration were passed down from Dr. William Maples at the C.A. Pound Human Identification Laboratory to a student of his at the Human Identification and Trauma Analysis Laboratory at Florida Gulf Coast University. The maceration process requires that the laboratory consider space, plumbing, exhaust control, and/or incubator costs, as well expenses related to the physical labor and time spent rendering the remains. Conversely, laboratory colonies have minimal physical plant requirements (i.e., reptile tanks and heating pads, substrate, and black-out materials that can range \$150–\$200 in initial supply costs) and low labor and time requirements for upkeep. Recent use of dermestids for cleaning skeletal remains have become more favorable, especially in museums and medical supply companies.<sup>1</sup>

*D. maculatus* are forensically significant and can assist forensic anthropologists in case analysis by cleaning delicate remains (e.g., neck organ blocks, subaqueous, or osteoporotic) with minimal to no damage and in a timely manner. In addition, beetles can reach into small, delicate, and easily fractured areas (ethmoid, lacrimals, infranasal conchae) to ensure thorough cleaning.<sup>2</sup> As their name implies, this species prefers dried—but not mummified—skin, muscles, ligaments, cartilage, and tendons. Although the beetles work “fast,” little research has been conducted to quantify their activities.

This study evaluated the optimum environment for the *D. maculatus* to render remains to bone. Past research has investigated the relationship between temperature and insect activity on the skeletonization process as part of estimations of Accumulated Degree Days (ADD), Postmortem Interval (PMI), or Time Since Death (TSD). This study builds upon the previous work by including the following variables: biomass (skeletal muscle and bone), insect abundance, and moisture content. Using three tanks, this study controlled for ambient temperature, light density, amount of biomass (g), and moisture (ppm). The samples drawn from Burmese pythons collected by the Conservancy of Southwest Florida (COSWFL) that were part of a larger study on these invasive species. The COSWFL personnel skinned, eviscerated, and disarticulated the pythons into 30cm–50cm sections. Wet (35%–40%) and dry (7%–14%) samples were placed into the colonies (3,000–15,000 beetles) with data being collected in two-hour intervals for the first six hours, then observed in six-hour intervals until all soft tissue was removed. With each observation, the weight (g), moisture, temperature, and pictures were taken for each of the samples using Vernier probes when applicable.

This study concludes that *D. maculatus* preferred the dry samples. The comparison of 400g–1,200g of python segments found that dry remains skeletonized significantly faster. The sample cleaned the fastest was two days (dry) and the slowest was nine days (wet). In no instance did the beetles observed eating the skeletal material. Consequently, this research highlights that *D. maculatus* colonies can be an important addition to forensic anthropology laboratories run by dedicated professionals.

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**Skeletonization, Burmese Python, *Dermestes Maculatus***



### **A52 Understanding Bone Diagenesis and Its Effects on Commingled Remains: The Potential and Hazards of Portable X-Ray Fluorescence (pXRF) Analysis**

*Kent M. Adamson, MSc\*, Middlesbrough, Tees Valley TS2 1AX, UNITED KINGDOM; Tim Thompson, PhD, Teesside University, Middlesbrough, Cleveland TS1 3BA, UNITED KINGDOM*

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**THIS ABSTRACT WAS NOT PRESENTED.**

## A53 DNA Preservation in Bone From a Multi-Individual Burial

Alexandra L. Emmons, MA\*, University of Tennessee, Knoxville, TN 37920; Sarah W. Keenan, PhD, South Dakota School of Mines & Technology, Rapid City, SD 57701; Jon Davoren, Lorton, VA 22079-2625; Jennifer M. DeBruyn, PhD, University of Tennessee, Knoxville, TN 37996; Amy Z. Mundorff, PhD, University of Tennessee - Anthropology, Knoxville, TN 37996-1525

**Learning Overview:** After attending this presentation, attendees will understand patterns of skeletal DNA variation in buried remains and the factors that affect these patterns. This presentation will increase attendees' knowledge of human decomposition processes, including bone decay, particularly as it relates to the soil environment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by furthering the knowledge of skeletal DNA degradation from buried environments.

There are three primary mechanisms to bone decay: (1) chemical deterioration of the organic phase of bone; (2) chemical deterioration of the inorganic phase; and (3) biodegradation. If the organic component degrades by either chemical or biological mechanisms, the mineral component, hydroxyapatite, becomes more vulnerable to environmental fluctuations and dissolution of the lattice structure.<sup>1</sup> This interdependence seems to suggest that greater porosity would allow for increased DNA degradation.<sup>1,2</sup> DNA sampling strategies and limited research has supported and emphasized this point, promoting the use of long, dense, cortical bones for DNA sampling and analysis. The preferential sampling of long bones has not only been related to DNA preservation but also to the ability to re-associate these bones in a commingled environment.<sup>3-6</sup> A growing body of research has indicated that small, cancellous bones outperform cortical bones in DNA quality and quantity.<sup>6-8</sup> The ease of sampling and reduced risk of contamination lends support to the use of smaller bones in certain cases, including disaster victim identification and may show utility in the case of a single individual burial.<sup>9,10</sup>

In a study looking at both intra-individual and inter-individual variation in human skeletal DNA preservation during surface decomposition, Mundorff and Davoren found that small cancellous bones, on average, yielded greater amounts of better quality DNA than larger, denser cortical bones, a pattern that held true up to 21 years postmortem.<sup>8</sup> The extent to which this pattern persisted in a burial remained unclear. The purpose of this research was to understand patterns of intra-individual and inter-individual skeletal DNA preservation in a multi-individual burial and how these patterns are shaped by burial factors, including soil geochemistry and biology. Three individuals were interred for four years prior to all analyses. Soil samples were collected during disinterment at four depths surrounding the bodies within the grave to look at changes in soil biology and geochemistry. To represent all bone types, a total of 49 bones per skeleton were sampled once for DNA analysis. To understand variation in human DNA quality and quantity from a single bone, 19 bones per individual were sampled from two sites, and three bones were sampled from three sites. Skeletal DNA was extracted, quantified, and globalfiler results were produced from each sampling site. Co-extracted bacterial and fungal DNA was also quantified, and bacterial communities were sequenced using targeted metagenomics of the V4 region of the 16S rRNA gene using Next Generation Sequencing (NGS).

Preliminary results indicate that the small, cancellous bones of the feet outperformed other bones in DNA yield. The third cuneiform, in particular, was among the top ten highest-ranking bones in terms of DNA yield across all three individuals. DNA yield varied by individual and depth within the grave, with the shallowest individual demonstrating the highest DNA yields. The feet exhibited the greatest variation in DNA yield across bone type and sampling site. Microbial communities were significantly different by individual and sample type (bone vs. soil) using a Permutational Analysis of Variance (PERMANOVA) on unweighted unifrac distances ( $n=263$ ,  $p=0.001$ ). Preliminary random forest models suggest that Amplified Sequence Variants (ASVs) (microbial taxa) influence human DNA yield when data from all individuals were combined ( $r^2=0.78$ ,  $p=0.00$ ).

This study provides a characterization of patterns of skeletal DNA preservation within a multi-individual grave. Results provide novel insights into skeletal DNA degradation that will likely inform skeletal DNA sampling strategies for buried remains.

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### **Skeletal DNA, Taphonomy, Microbial Ecology**



### A54 Recognizing Transplanted Allograft Bone in Forensic Anthropological Cases

*Angi M. Christensen, PhD\*, FBI Laboratory, Quantico, VA 22135; Louis Jares, BS, MTF Biologics, New Braunfels, TX 78130*

**Learning Overview:** After attending this presentation, attendees will understand how to recognize transplanted bone if it presents in a forensic anthropological context.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing forensic anthropologists regarding the likely appearance of transplanted bone in a forensic context, so it is not mistaken for commingling.

Given that structural allograft bone transplants are now routinely used to treat skeletal traumas and cancers, it is possible that bone grafts may be observed in forensic anthropological cases. Because these transplants involve large portions of donated cadaveric (non-native) bone, such cases could potentially complicate forensic anthropological investigations by mimicking the presence of a second individual in terms of bone morphology and size. However, they may also provide significant leads for personal identification. Here, an overview of structural allograft bone transplantation is provided to make forensic anthropologists aware of how these cases may look in forensic anthropological contexts.

Bone transplants have been in use for more than 130 years and are routinely used in association with treatment of severe bone loss due to trauma, orthopedic implant procedures, and oncology. Bone transplantation most often uses human donor grafts. These grafts are sometimes non-structural and small-scale such as bone chips, powders, or pastes which are typically used to enhance or improve fusion or healing. They may also be large-scale structural transplants that take the form of bone portions or even entire bones that are typically used for limb salvage or to restore weight-bearing function. Autografts use skeletal material procured from the individual receiving the graft, while allograft bone is procured from one or more deceased individuals, depending on the complexity and scope of the surgical procedure.

Once implanted, the graft recipient effectively possesses both native and non-native skeletal material. Although efforts are typically made to use size-appropriate grafts, the transplanted bone may still be somewhat morphologically and metrically different from the native antimeres of the recipient. This means that in the event a recipient of a structural bone allograft becomes the subject of a forensic anthropological examination, skeletal analysis could be affected if the transplant is not recognized and is instead mistaken for commingling based on these dissimilarities in antimeres.

One advantage of a bone transplant in a forensic anthropological case is that the surgery will likely be apparent due to either bone remodeling or the association of a surgically implanted device. The healing/remodeling of bone should be apparent as an antemortem process that may be linked to an injury and/or surgery. Evidence of surgery may be helpful in narrowing the pool of possible missing persons to whom the remains may belong. Surgically implanted devices can similarly help search for possible missing persons or can sometimes be used to corroborate identity. Knowledge of the appearance of bone transplants may help forensic anthropologists in recognizing alterations and hardware associated with this procedure and help provide significant leads in identifying the remains.

It is unknown how often (if ever) bone transplants have been observed in forensic anthropological cases. Due to the presence of native and non-native bone in the same skeleton, it is possible that transplanted bone could result in metrically and morphologically dissimilar antimeres and mimic commingling, thereby misleading the analysis. However, awareness of this process and its appearance should help in recognizing when a bone transplant may be involved and mitigate such complications. This awareness may be enhanced through increased and continued communication and collaboration between medicolegal experts and those in the tissue transplant community.

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#### Forensic Anthropology, Bone Transplant, Commingling



### A55 Inferring Species Origin Through Virtual Histology: A Comparison of Third Metapodials From *Homo Sapiens* and *Ursus Americanus* Using Micro-Computed Tomography

Hannah Stephen, BS\*, University of Akron - Department of Biology, Akron, OH 44325; Reed A. Davis, MSc\*, University of Akron - Department of Biology, Akron, OH 44325; Janna M. Andronowski, PhD, University of Akron, Akron, OH 44325-3908

**Learning Overview:** The goals of this presentation are to: (1) describe the microstructural differences among human and black bear metacarpals and metatarsals using a non-destructive and volumetric 3D approach, and (2) provide microarchitectural data that will aid species identification efforts when bone fragments are discovered in a forensic context.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing 3D parameters to the study of bear bone microstructure, providing additional information that will aid in the identification of morphologically similar human and non-human remains.

The gross similarities between human hand and foot bones and bear paws have been well documented. Macroscopic skeletal analyses provide insight into species origin when whole bones are recovered but are frequently rendered inapplicable when bones are fragmented. In these scenarios, histological techniques are often applied. However, specific research focusing on the quantification of bear bone microstructure remains scarce in the literature.

It is hypothesized that 3D analysis of bear cortical bone microstructure provides a more representative and accurate means to infer species origin from fragmented metapodials.

Methods included visualizing and quantifying bone microstructural parameters using micro-Computed Tomography (micro-CT). Black bear (*Ursus americanus*) metapodials were loaned from the Vertebrate Zoology Department at the Cleveland Museum of Natural History. Human bone specimens were obtained from the University of Akron's skeletal teaching collection housed in the Department of Biology. The third metacarpals and metatarsals from mature black bears ( $n=5$ ) and mature humans ( $n=5$ ) were assessed using 3D analyses. All selected bear and human specimens did not display visible signs of pathology and were from the left side of the body.

Micro-CT experiments were conducted using a SkyScan 1172 laboratory X-ray system at the Surface and Optical Analysis Facility at the University of Akron's Polymer Innovation Center. A source spot size of 5.5 $\mu$ m and 8.83 camera pixel size were used. A rotation step of 0.20 degrees, X-ray settings of 100 kV and 100  $\mu$ , 0.50 frame averaging, and a combined aluminum and copper filter were applied to obtain 1,224 projections spanning 180 degrees of rotation. Prior to all scans, flat and dark-field projections were collected to correct for noise in the detector and X-ray beam.

Micro-CT projections were reconstructed using NRecon 1.6.10.2. Cylindrical Volumes of Interest (VOIs) were identified within each bone sample. Image stacks were cropped and analyzed using CTAnalyser 1.15.4.0. The variables measured included: Total VOI Volume (TV), total Canal volume within VOI (Ca.V), Canal Number (Ca.N), average Canal Diameter (Ca.Dm), and Cortical porosity (Ca.V/TV). Statistical analyses were accomplished using SPSS 23.0 statistical software. To assess whether species and element have a significant effect on Ca.V/TV, Ca.N, and Ca.Dm, independent  $t$ -tests (comparisons between species) and paired  $t$ -tests (comparisons within species) were performed at a significance of  $p \leq 0.05$ .  $r^2$  values were calculated to assess the fraction of variance present between the variables compared. The following qualitative features were further described: bone composition (e.g., woven, fibrolamellar, Haversian), osteon banding, and resorptive spaces.

Between-species  $t$ -tests revealed that Ca.N significantly differed between human and bear metacarpals ( $t_{0.05(2),5}=4.862$ ;  $p < 0.05$ ) and metatarsals ( $t_{0.05(2),5}=8.416$ ;  $p < 0.05$ ), as did Ca.Dm between human and bear metacarpals ( $t_{0.05(2),5}=-2.130$ ;  $p < 0.03$ ) and metatarsals ( $t_{0.05(2),5}=-2.568$ ;  $p < 0.03$ ). No significant results were found for Ca.V/TV, though the  $r^2$  values for the metacarpal measures between human and bear indicated a moderate effect size ( $r^2=69.1$ ;  $p < 0.05$ ). Within-species  $t$ -tests revealed no significance difference between the paired cortical parameters for either the human or bear specimens. However, measures of the metacarpals between human and bear specimens indicated a medium effect size ( $r^2=27.0$ ;  $p < 0.211$ ). Ca.N and Ca.V/TV differed among human metacarpals and metatarsals, with the parameters being greater in the metatarsals. Qualitative features including osteon banding and resorption spaces were more prevalent in the bear metacarpals and metatarsals. Plexiform bone was observed in the bear metapodials only.

The 3D data for this study were obtained non-destructively and reveal the usefulness of laboratory micro-CT as a diverse and novel tool for the anthropologist. The volumetric nature of this approach demonstrates that it is possible to differentiate fragmented bear and human metapodials, both quantitatively and qualitatively.

#### Black Bear, Micro-Computed Tomography, Metapodials



### A56 The Scavenging Patterns of Feral Cats on Human Remains in an Outdoor Setting

Sara N. Garcia\*, Grand Junction, CO 81507; Alexander J. Smith, BA\*, Grand Junction, CO 81502; Christiane Baigent, MSc, Forensic Investigation Research Station, Grand Junction, CO 81501; Melissa A. Connor, PhD, Colorado Mesa University, Grand Junction, CO 81501-3122

**Learning Overview:** After attending this presentation, attendees will better understand the pattern of feral cat scavenging on human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing examples of feral cat scavenging to help distinguish between postmortem artifact and peri-mortem injury that may be associated with cause of death.

Scavenging of human remains by the Felidae family is documented, but case reports are rare. Rather than scavenge, Felids prefer to hunt. When scavenging occurs, Felids prefer to exploit fresh tissue across several days.<sup>1</sup> In the only published case of bobcat scavenging in North America, the bobcat focused on the tissues of the arms, hips, and thighs. However, in this case, the remains were autopsied, resulting in penetrating trauma and visceral loss that may have affected the pattern of consumption.

Scavenging by domestic cats (*Felis catus*) is also rarely reported.<sup>1</sup> The rarity of domestic cat scavenging may be explained by two well-documented selection mechanisms rooted in the sensory characteristics of food sources: (1) neophobia, the rejection of foods not previously encountered; and (2) “the novelty effect,” a preference for previously familiar foods that are spatially or temporally limited in availability.<sup>2</sup> Additionally, the smaller size, strength, and bite force of *Felis catus* may preclude the ability to open the human thoracic cavity, and so their pattern of scavenging may differ from the pattern observed in larger cats.<sup>1</sup>

Two separate incidents of feral cat scavenging of human remains occurred at the Forensic Investigation Research Station (FIRS), Whitewater, CO. The outdoor facility is fenced to keep out large scavengers but does not restrict the access of small mammals and avian scavengers. In both cases, the remains were refrigerated between time of death and placement.

**Case 1:** A 79-year-old female was placed 13 days after death. Subcutaneous needles were inserted as part of an unrelated research study and the decomposition accelerated at the needle insertion sites. Scavenging began approximately five days after placement, near two of the needle insertion sites and presented as small circular defects on the distal upper arm, proximal to the elbow. A game camera photographed a striped cat scavenging the donor. The cat consumed tissue from the left arm and adjacent chest. To stop the scavenging, a cage was placed over the donor. The cage deterred the cat and when the cat had not appeared for approximately one week, the cage was removed. Shortly thereafter, the cat returned to the body. The cat did not demonstrate an interest in any of the 40 adjacent donors. The cat continued to scavenge almost nightly for approximately 33 days.

**Case 2:** A 70-year-old autopsied male was placed approximately 11 days after death. Scavenging began approximately six days after placement at the lateral left shoulder along the autopsy incision, the lower lateral abdomen, and the proximal arm. A game camera observed a black cat scavenging the donor. No attempt was made to prevent scavenging. The cat returned most nights for 16 nights, then returned for two nights one week later. On each night, the cat visited the body multiple times. The same cat returned to the body for approximately 25 days.

In the few reported cases of indoor domestic *Felis catus* scavenging, the target was the face (primarily the mouth and nose), hands, and feet.<sup>3</sup> In both cases reported here, feral cats targeted the arms and chest. Secondary areas of interest included the abdomen. In both cases, patterns of feral cat scavenging more closely paralleled the pattern of bobcat scavenging than the domestic *Felis catus*. Differences in feeding behavior between domestic and feral cat groups are reported and are most often attributed to both neophobia, and the need for reflexivity in the feral cat diet as they work to meet nutritional needs.<sup>2</sup> The novelty effect may partly explain the propensity for a specific human tissue source.

Tissue damage due to postmortem scavenging can be confused with peri-mortem trauma. Recognizing the scavenging patterns of a variety of animals is important for investigators to determine the origin of the damage.

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#### Cat Scavenging, Taphonomy, Postmortem Scavenging



### A57 Positive Identification Via Frontal Sinus Morphology: A Geographic Information Systems (GIS) Approach

Jenna M.S. Watson, MA\*, University of Tennessee, Department of Anthropology, Knoxville, TN 37996-1525

**Learning Overview:** After attending this presentation, attendees will understand the application of Geographic Information Systems (GIS) software for establishing positive identifications from antemortem and postmortem frontal sinus radiographs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an example of a novel application of GIS software, arcGIS® and ArcMap®, for the purposes of human identification.

Visual comparison of Antemortem (AM) and Postmortem (PM) frontal sinus radiographs has been used to establish positive identifications when other methods, such as fingerprint and dental matching, are not available. However, observer experience continues to influence method performance.<sup>1</sup> In order to address the need for more quantifiable methods in human identification, this research explored the utility of ArcMap® for frontal sinus identification.<sup>2</sup> The main objective of this study was to assess the utility of ArcMap® and its spatial analyst tool, Similarity Search, for identifying a frontal sinus match from radiographs that were collected for a previous study.<sup>2,3</sup> It was hypothesized that Similarity Search would be able to: (1) correctly identify a frontal sinus match based on area and perimeter values; and (2) provide practitioners with a quantifiable and reproducible method for positive identification using cranial radiographs.

Radiographs of 50 male and 50 female donors associated with the University of Tennessee William M. Bass Donated and Forensic Skeletal Collections were digitized using a Diagnostic Pro-Edge Scanner, organized into randomly selected test groups that contained one PM and ten AM radiographs, uploaded into ArcMap® 10.5, and digitized into two-dimensional polygons with area and perimeter values.<sup>2</sup> Similarity Search was instructed to: (1) compare each AM polygon to the PM polygon for that group by area and perimeter values; then (2) rank each AM polygon based on similarity to the PM polygon and calculate a corresponding similarity index value. Hierarchical cluster analysis was used to determine a similarity index value range. Inter- and intra-observer variation was assessed indicating low variation within and among observers and the one-way Analysis of Variance (ANOVA) displayed no significant difference among observers for area or perimeter ( $p=0.935$ ,  $p=0.906$ ). Similarity Search correctly identified the true match polygon for 31/50 male groups (62%) and 36/50 (72%) female groups. Based on the cluster analysis, the range of similarity index values for females is 0–11.56, and for males is 0–5.51.

The results of this study demonstrate that Similarity Search can correctly identify a match for males and females with 62% and 72% accuracy, respectively. Results indicate that this is a user-friendly and replicable method with promising initial results despite limited parameters. However, future research that includes additional characteristics beyond area and perimeter will likely improve performance and increase reliability of this method. For example, the use of zonal geometry or the inclusion of a shape analysis algorithm should be explored. Moreover, the inclusion of 3D images (e.g., Computed Tomography (CT) scans) in place of radiographs may resolve issues encountered with ArcMap®, including orientation, scale, and clarity.

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#### Frontal Sinus, Geographic Information Systems, Human Identification





### A58 The Identification of United States Casualties by the Central Identification Unit in Kokura, Japan

Alexander F. Christensen, PhD\*, DPAA, Joint Base Pearl Harbor-Hickam, HI 96853

**Learning Overview:** After attending this presentation, attendees will better understand how the United States military made identifications of war casualties before the development of DNA-based methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining how certain we can be regarding the historical identification of United States war casualties.

On March 23, 1951, the USS *General George M. Randall* arrived in Oakland, CA, carrying the remains of 57 United States servicemembers who had died in the Korean theater. Of these, seven were recent casualties whose remains went directly from the hospital to the mortuary. The others had been buried the previous fall in temporary cemeteries in South Korea prior to their exhumation and forensic identification. In every previous American conflict, casualties had been buried near where they fell. After both world wars, remains were exhumed and identified, then either repatriated to the United States or reburied overseas, depending upon family preference. But in the fall of 1950, the United States military decided to repatriate all remains to the United States while the conflict was still ongoing. This policy is still in place, and the *General Randall* was unfortunately only the first of many transports to come. By the date of its arrival in California, more than 17,000 United States servicemembers had died in Korea, and the last regular shipment of remains home was in 1956.

As part of an ongoing study, the means used to identify more than 600 casualties between 1951 and 1956 have been tabulated. Throughout this period, all remains of casualties who did not die under direct medical supervision were processed by the United States Army Central Identification Unit (CIU) at Camp Kokura, Japan. Identifications relied upon dental and anthropological analyses, personal effects, and other circumstantial evidence, and, when available, fingerprints (which were sent to the Federal Bureau of Investigation (FBI) for comparison with their dataset of service members). One of the individuals on the *General Randall* was the fifth case processed by the CIU, on January 29. His remains were identified on February 22 based on his identification tag, and the determination that the dental status, height, and hair color of the remains all agreed with his personnel records; confirmation of a fingerprint match was received from the FBI on March 9.

Of the casualties in this sample, 408 were identified in 1951, and this subsample was the focus of analysis. These identifications were largely based on one-to-one comparison: 306/408 were received with name associations (254 based on identification tags, 52 based on personal effects, other identification media, or witness statements), and the remains were compared with a single individual's antemortem records to confirm the identification. When possible, fingerprints were used to make positive identifications, and the FBI confirmed the identifications of 92/306. For the 102 without name associations, or Unknowns, larger comparisons based on recovery date and location were generally necessary, and 74/102 were confirmed by the FBI.

Every identification memo addressed the dental remains, even if just to say (in nine cases) that dental comparison was not feasible because either dental remains or antemortem records were absent. Forty-seven cases were judged "extremely favorable" and 53 "very favorable;" other standards were "favorable," "not unfavorable," "applicable," "very applicable," and "in agreement." When discrepancies were noted, often a dental officer wrote a memo explaining why he felt those discrepancies could be discounted.

Estimated stature was used in 395/408 identifications. In most of the others, remains were too fragmentary. In 13 cases, differences of 2"-4" between antemortem and postmortem statures were explained by growth since enlistment. Race of the remains was only addressed in 150/408 cases; this is probably because the majority of casualties in this sample were European-American, so ancestry was of little discriminative value. Similarly, age was only addressed in 170/408 cases, but for a different reason: many of the remains processed in the spring of 1950 were only semi-skeletonized, and the anthropologists at the CIU would not estimate age if they could not observe the pelvis or any epiphyses. In 25 cases, matches were made between antemortem fractures and surgical procedures documented in medical records and those observed in the remains; in six cases, statements were included explaining why it was reasonable that a documented fracture was no longer observable.

#### Military Casualties, Biological Profile, Dental Identification



## A59 Interactive Resources for Craniofacial Identification

*Terrie Simmons-Ehrhardt, MA\**, Virginia Commonwealth University, Richmond, VA 23284; *Anthony B. Falsetti, PhD*, George Mason University, Fairfax, VA 22030; *Catyana R. Falsetti, MFS*, Fairfax, VA 22030; *Christopher J. Ehrhardt, PhD*, Virginia Commonwealth University, Richmond, VA 23284

**Learning Overview:** After attending this presentation, researchers and practitioners of craniofacial identification methods will be familiar with free and/or open-source resources for interacting with 3D craniofacial reference data generated from Computed Tomography (CT) scans in both digital and physical formats.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing an interactive 3D craniofacial reference dataset and detailed workflows for viewing and processing 3D models, which will enhance training/education efforts by increasing exposure to a large range of craniofacial variation, facilitate collaborative efforts between researchers and practitioners of craniofacial identification methods, as well as initiate discussions for standardizing reference data.

3D digital models of skulls and faces were generated from de-identified, publicly available CT scans at The Cancer Imaging Archives website. Landmarks were collected to describe facial feature dimensions and positions relative to bone landmarks, for comparison to traditional facial approximation guidelines, and to derive new predictive regressions or positional predictions. The dataset has been transformed to a common orientation and coordinate system to allow viewing along aligned planes (Frankfurt Horizontal, coronal plane, and midsagittal plane), resulting in distances and positions between landmarks across a single axis, two axes, or all three axes, that are translatable to 2D and 3D methods of facial approximation, as well as craniofacial superimposition.

A workflow for dense Facial Tissue Depth Mapping (FTDM) has been developed with open-source software, which utilizes the geometric relationships between the face mesh and skull mesh to calculate distances for every face point and colorizes and stores the depths within the face and skull meshes as a “vertex quality.” The workflow can be applied to any 3D bone-skin models derived from CT or cone-beam CT. The resulting output is a set of red-green-blue colorized Polygon File Format (PLY) meshes and point clouds from which tissue depth information can be read and interacted with in 3D in Meshlab. The FTDMs have also been “split” into smaller models representing 1.0mm-depth increments to facilitate viewing of a specific depth over the entire face and/or skull or across multiple individuals.

Because of the steep learning curve associated with 3D programs, an .html viewer was developed that opens within a web browser from local files, using the open-source 3DHOP platform. The interactivity is more straightforward and less daunting than available software packages, with clear icons and sliders with specific functions to adjust views (direction, transparency, and color), place points, collect measurements, and even clip skin away from the skull along the aligned planes. The ability of practitioners and researchers to interact with a large dataset of digital faces and skulls in 3D, by viewing transparent skin over the bone in correct anatomical position, provides an opportunity for enhanced training and a more objective understanding of the relationship of the face to the underlying craniofacial skeleton.

Further, all Stereolithography (STL) files are 3D printable and can be further clipped and edited for 3D printing to use physical models in training, classroom, or workshop settings. Full skull models could be printed at actual size for sculpting exercises. 3D Slicer’s “EasyClip” tool allows cutting at specific landmark coordinates (x-, y-, or z-). Face shells generated during FTDM can be clipped and “thickened” in Meshmixer to the minimum tissue depth to allow printing of skin over bone. Models can be generated and printed for training on specific facial features or to simultaneously visualize bone and skin, including models with faces clipped along the midsagittal line to reveal bone features on one side and corresponding facial features on the opposite side. Profile prints, mimicking radiographs, could be generated from a midsagittal profile (by clipping 1mm–2mm on either side of nasion) of the original face model, resulting in “printed skin” and bone as empty space. Such a model set would allow a detailed study of the nose in profile, utilizing examples from numerous individuals, easily exposing trainees/practitioners to a wide range of anatomical variation, both digitally and physically.

The digital and physical learning environments and increased exposure to a large range of craniofacial variation may lead to more anatomically accurate representations of the human face, establish new venues for collaboration between researchers and practitioners, and ultimately contribute to large, standardized reference datasets for craniofacial identification efforts.

*Funding provided by a National Institute of Justice Award.*

### **Craniofacial Identification, Facial Approximation, 3D**

### A60 What Can Forensic Proteomics Tell Us About Biological Age and Postmortem Interval (PMI) Estimation?

Noemi Procopio, PhD\*, Northumbria University, Newcastle upon Tyne NE1 8ST, UNITED KINGDOM; Anna Williams, PhD, University of Huddersfield, Huddersfield, West Yorkshire HD1 3DH, UNITED KINGDOM; Andrew Chamberlain, PhD, The University of Manchester, Manchester M13 9PT, UNITED KINGDOM; Michael Buckley, PhD, The University of Manchester, Manchester M1 7DN, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will understand both the state of the art in forensic proteomics, with its applications to biological age and PMI estimation, and a novel finding of potential protein biomarkers for Age At Death (AAD) and PMI estimation from skeletonized remains, an innovative and ground-breaking application in the forensic field.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showcasing a novel application of proteomics to estimate PMI and AAD with increased success.

One of the most debated themes in forensic anthropology is the estimation of PMI as well as the approximation of the AAD from skeletal remains; despite the presence of distinct analytical and morphological methods to address these aims, their applicability on heavily decomposed bodies or skeletonized remains is strongly limited and suffers from poor accuracy.<sup>1-3</sup> Due to the well-known use of proteomic methods to evaluate aging and decay phenomena for archaeological purposes, this study applies proteomics to forensic applications, looking for new biomarkers in pig skeletal remains that could address AAD and PMI from a previously unexplored perspective.<sup>4</sup>

To achieve these goals, two separate studies were performed. The first compared porcine skeletal remains from five different-aged animals to look for intra-bone and inter- and intra-skeletal proteomic differences, to evaluate potential biomarkers for AAD estimation. Bones were sampled, their proteomes extracted using a protocol that minimizes laboratory-induced decay and were submitted to Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-Orbitrap-MS/MS) analysis. For the second part of this study, four piglets of similar age were experimentally buried, and their bones collected at selected time points (one to six months PMI). Samples were then subjected to the same treatment mentioned above but focused on the evaluation of proteins leaching into the surrounding soil, as well as proteome postmortem decay via the study of post-translational modifications as a means to estimate PMI.

Results demonstrated greater intra-bone rather than inter-bone and intra-individual proteomic differences, and overall higher data reproducibility was obtained sampling the midshaft of long bones (tibiae) than their epiphyses. When using tibiae midshaft to compare proteomes among different-aged pigs, a bone protein, fetuin-A, was observed to be significantly different in terms of its relative abundance, which was negatively correlated with the biological age of the individual; this phenomenon is one example by which forensic proteomics could provide alternative means to evaluate the AAD from bone samples. When investigating proteomic differences between carcasses with different PMIs, it was possible to observe a gradual leach of several groups of proteins from bones as a function of PMI, starting with serum and muscle proteins that were the first to decrease in abundance with prolonged burials. Looking specifically at post-translational protein modifications, biglycan deamidation ratios appeared to increase with protracted PMIs in a statistically significant way. Further studies may validate the use of this protein as new potential biomarker for PMI estimation ranging from one to six months and beyond.

Overall, these studies set the baseline for increasing the awareness of the potential application of proteomics to forensic science, allowing the development of future works to extend the dataset to a wider range of biological ages and also to prolonged PMIs. Further studies on human samples will be required to corroborate these results, to allow their application to real forensic casework.

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#### AAD Estimation, PMI Estimation, Proteomics

### A61 Using Stable Nitrogen Isotope Ratios From Human Muscle Tissue for Postmortem Interval (PMI) Estimation

Melanie M. Beasley, PhD\*, Knoxville, TN 37996-0720; Julie Lesnik, PhD, Wayne State University, Detroit, MI 48202; Hayden McKee, MSc, Knoxville, TN 37917; Dawnie W. Steadman, PhD, University of Tennessee, Knoxville, TN 37996

**Learning Overview:** After attending this presentation, attendees will understand how changes in stable nitrogen isotope ratios ( $\delta^{15}\text{N}$ ) of decomposing human muscle tissue may aid in the estimation of the PMI.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel application of stable isotope analysis of  $\delta^{15}\text{N}$  ratios of decomposing human muscle tissue to use as a useful alternative to traditional PMI estimation techniques. Furthermore, this study explores the use of  $\delta^{15}\text{N}$  ratios of maggots feeding on decomposing muscle tissue to assess if a systematic trophic level enrichment can be used for a PMI estimate after identifiable muscle tissue is no longer present.

To test how  $\delta^{15}\text{N}$  ratios can be used as a PMI estimate, it is key to understand two basic principles: (1) what a  $\delta^{15}\text{N}$  is measuring; and (2) what is happening to nitrogen during muscle tissue decomposition. Nitrogen has two stable isotopes with different masses, a heavy ( $^{15}\text{N}$ ) and a light ( $^{14}\text{N}$ ) isotope. In a chemical reaction, the isotopes with different masses will react at different speeds, resulting in a preferential selection of one isotope ( $^{14}\text{N}$ ) compared to the other ( $^{15}\text{N}$ ). During decomposition of muscle tissue (i.e., putrefaction), the breakdown of amino acids produces a variety of volatile compounds, including ammonia ( $\text{NH}_3$ ) and lesser amounts of two other nitrogenous gases, cadaverine ( $\text{NH}_2(\text{CH}_2)_5\text{NH}_2$ ) and putrescine ( $\text{NH}_2(\text{CH}_2)_4\text{NH}_2$ ). As putrefaction progresses, the chemical reactions to produce the nitrogenous gases would preferentially incorporate the light isotope ( $^{14}\text{N}$ ) because it will react faster in a chemical reaction, resulting in an enrichment of the heavy isotope ( $^{15}\text{N}$ ) in the remaining muscle tissue. The impact to  $\delta^{15}\text{N}$  ratios as putrefaction progresses would be higher values compared to the  $\delta^{15}\text{N}$  ratios of the fresh muscle tissue.

This research was conducted at the Anthropology Research Facility in Knoxville, TN, an outdoor laboratory for the study of human decomposition. A total of 20 donors were enrolled in the study, and muscle and associated maggot samples were collected for as long as each were present on a donor. Donors were placed on the surface in two trials with ten donors each in the winter and summer season to test differential seasonality effects to the method as a PMI estimator. The  $\delta^{15}\text{N}$  ratios from the muscle and maggot samples were analyzed. The raw  $\delta^{15}\text{N}$  ratios are not of interest because each donor has a different baseline value. To normalize the assessment of change in  $\delta^{15}\text{N}$  ratios, the  $\Delta^{15}\text{N}$  was calculated and compared ( $\Delta^{15}\text{N} = \delta^{15}\text{N}_{\text{Day } X} - \delta^{15}\text{N}_{\text{Day } 1}$ , with Day 1 as the initial fresh tissue sample).

Initial results indicate that alteration of  $\delta^{15}\text{N}$  ratios in decomposing muscle tissue can be used for PMI estimates when identifiable muscle tissue is still present. The general pattern follows expectations that  $\Delta^{15}\text{N}$  values increase as putrefaction progresses, with as much as a 3.0% increase in muscle tissue values. The winter trial donors had identifiable muscle tissue for up to three months, while the summer trial donors had muscle tissue for only two weeks. When the winter trial  $\Delta^{15}\text{N}$  values are grouped in 20-day increments, there is no significant difference between the initial Day 1 sample and the Day 2–20 group ( $p=0.258$ ) or the Day 21–40 group ( $p=0.197$ ). However, past Day 40, there is a significant difference between the initial Day 1 sample and the Day 41–60 group ( $p=0.003$ ) or the Day 61–80 group ( $p=0.000$ ). The associated maggot  $\Delta^{15}\text{N}$  values will eventually be combined with the muscle data to assess the usefulness of using live maggots found associated with a body at a crime scene to estimate PMI after identifiable muscle tissue is no longer available.

This study revealed that change in the  $\delta^{15}\text{N}$  ratios of decomposing muscle tissue can be used as an alternative method for PMI estimation, especially in the winter months when identifiable muscle tissue can be retained on a body for months. This East Tennessee pilot study indicates that the significant influence to when  $\Delta^{15}\text{N}$  values start to increase is associated with a shift to increasing temperatures when putrefaction progresses faster. This initial study indicates there is value in the method as a PMI estimate for recently deceased individuals, but further work is needed in other environmental contexts.

#### Stable Nitrogen Isotope Ratios, Postmortem Interval Estimation, Muscle Tissue Decomposition



### A62 The Human Cadaver Decomposition Island and the Vegetation Regrowth Interval

*Haeli Kennedy, Sam Houston State University, Huntsville, TX 77341; Jasmine Garcia, Applied Anatomical Research Center, Huntsville, TX 77341; West Ryan, Huntsville, TX 77341; Jacqueline A. Aitkenhead-Peterson, PhD, 2474 TAMU - Department of Soil & Crop Sciences, College Station, TX 77843; Joan A. Bytheway, PhD\*, Sam Houston State University, Huntsville, TX 77341-2506*

**Learning Overview:** After attending this presentation, attendees will have a clear understanding of what a surface human Cadaver Decomposition Island (CDI) looks like with and without scavenging interference and soil chemical composition results obtained throughout the study. Attendees will understand that the CDI is present months after the remains are removed and the concentration of soil nutrients fluctuate during the decomposition process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing awareness to those entities participating in search and rescue efforts to not only focus on looking for the skeletal remains of an individual but to also look for the CDI and collect soil samples. Both the CDI and soil nutrient composition are useful pieces of evidence.

Typically, during the late phase of the Early stage of human decomposition, fluids of the body, formed during autolysis, hydrolysis, and by other chemical and bacterial actions, leach into the soil under and around the body producing a CDI. The fluid contains high concentrations of organic matter and microorganisms, eradicating any vegetation adjacent to and under the body. When the soil pH and nutrients in the upper soil horizons appear to return to a level similar to the original state, translocated nutrients in the soil cause native vegetation, and flora not indigenous to that area, to flourish. Previous research observing regrowth of vegetation has been conducted using *Sus scrofa*, although no literature to date has recorded the vegetation regrowth of a human CDI.<sup>1</sup>

In 2013 and 2018, studies were conducted at the Applied Anatomical Research Center (AARC) in Huntsville, TX. The studies demonstrated that the CDI is greatest under the trunk, reduced under the head, and further reduced under the limbs. The studies documented the timing of the formation of the CDI as well as the timing of the return of vegetation around and within the CDI. In 2013, 16 subjects were placed, and, in 2018, 4 additional subjects were placed with the inclusion of soil sampling throughout the study. For both studies, subjects were in similar environmental conditions. After collapse of body cavities and formation of the CDI, caged subjects were removed from their CDI at predetermined intervals of zero, two, and four weeks after leaching. Cages remained over the CDIs and daily observation occurred until regrowth within the CDI formed. For 2013 subjects removed at zero weeks (the day after CDI formation appeared complete), regrowth within the CDI occurred after 131 days. For 2013 subjects removed at two weeks after CDI formation, regrowth occurred after 197 days. Most of the CDI for subjects accessible to scavenging had formed under the trunk of the body prior to being moved around by scavengers. However, the CDI of the limbs and head could not be clearly identified after movement of the body by scavenging had smeared the soil. Bodies were pulled away from the major part of the CDI by scavengers but remained at the periphery throughout the study. Between 90 and 207 days, vegetation began to appear in the CDIs of scavenged subjects. The trunks of the bodies that had minimal movement by scavenging prolonged the CDI's composition.

Results demonstrate that in the piney woods region of southeast Texas, if a decomposing body can leach the majority of its fluid in an area, prior to extensive scavenging, vegetation regrowth may take approximately 90–200+ days to appear. In search and rescue efforts for missing individuals presumed dead, a CDI may be detectable three to seven months after death even if the body has been displaced from its original location. 2018 study observation and soil chemistry results will be presented at the American Academy of Forensic Sciences (AAFS) meeting in February 2019.

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#### Forensic Anthropology, Taphonomy, Soil Composition



### A63 Longitudinal Comparison of the Megyesi and Moffatt Total Body Scoring (TBS) Methods for Estimating the Postmortem Interval (PMI)

Derek A. Boyd, MA\*, University of Tennessee, Knoxville, TN 37996; Kelly Sauerwein, PhD, University of Tennessee, Knoxville, TN 37996; Angela M. Dautartas, PhD, University of Tennessee, Knoxville, TN 37996-1525

**Learning Overview:** After attending this presentation, attendees will better understand the limitations of relying on temperature-based predictive models for estimating the PMI.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting best practices regarding the use of these PMI estimation methods in forensic anthropological research and casework.

Knowledge of the PMI is crucial to medicolegal death investigation, but variation in human decomposition prevents practitioners from producing accurate and informative time-since-death estimates. To address this issue, Megyesi et al. retrospectively developed a regression-based method for predicting the PMI, termed the Total Body Scoring (TBS) method, that quantified the relationship between gross anatomical changes and the amount of accumulated temperature required to produce these changes.<sup>1</sup> Since its initial publication, the TBS method has had variable success estimating the PMI for decedents from a variety of different climates and depositional contexts. Moffatt et al. recently published a modified formula and methodology for PMI estimation, termed the Modified TBS (MTBS) method, that purportedly performs better than the TBS method.<sup>2</sup> The objective of this study was to independently and longitudinally validate the accuracy and precision of the MTBS method using 25 decedents who were received as donors by the University of Tennessee William M. Bass Body Donation Program between March 2014 and April 2017.

Temperature data (°C) were collected from Tinytag® data loggers and TBSs were obtained through daily observations (up to 112 days after placement) performed in person at the Anthropological Research Facility. Some donors ( $n=10$ ) were scored assigned TBSs by multiple observers. To include these data, the TBSs for each donor were averaged across observers because observer disagreement was negligible (Cronbach  $\alpha > 0.90$ ). All TBSs were input into the Megyesi et al. equation to produce daily Accumulated Degree Day (ADD) point estimates and associated 95% prediction intervals for each donor.<sup>1</sup> To facilitate validation of the MTBS method, each TBS was then reduced by three and input into the Moffatt et al. modified equation to produce ADD point estimates.<sup>2</sup> The associated 95% prediction intervals for each modified TBS were then taken from the data table provided by this study. Preliminary results demonstrate that the TBS method was able to more accurately estimate ADD than the MTBS method for the data set used here; however, prediction intervals were smaller for the MTBS method than for the TBS method, indicating higher precision for the former. Additionally, significant differences in ADD point estimates were observed between both methods investigated in this study ( $p < 0.0001$ ).

These findings suggest that, despite large prediction intervals, the TBS method is better at predicting ADD in East Tennessee; however, these results importantly demonstrate that there are instances in which neither method is able to accurately estimate time since death, regardless of statistical modifications, supporting recent studies that argue for continued caution in utilizing temperature-based predictive models that are based on gross presentation of decedents. It is strongly recommended that researchers and practitioners in other environments validate the reliability of the MTBS method in relation to the TBS method before considering the statistical modifications provided by Moffatt et al, and that future research projects consider longitudinally evaluating the efficacy of methods like those presented here across multiple seasons.<sup>2</sup>

#### Reference(s):

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#### Time Since Death, Accumulated Degree Days, Human Decomposition



### A64 The Effects of Hydrochloric Acid (HCl) on Decomposition: Evidence of Preferential Destruction to the Head and Extremities

Dayanira Lopez, BS\*, Florida Gulf Coast University, West Palm Beach, FL 33405; Heather A. Walsh-Haney, PhD, Florida Gulf Coast University, Fort Myers, FL 33965-6565; Laura Frost, PhD, Florida Gulf Coast University, Fort Myers, FL 33965; Brian Johnson, PhD, Florida Gulf Coast University, Fort Myers, FL 33965

**Learning Overview:** After attending this presentation, attendees will understand the effects of HCl on human remains, specifically demonstrating preferential destruction of the head and extremities over the torso, as replicated in a study using a pig (*Sus scrofa*) to compare the pattern of dissolution. Finally, the effect that estuarine water has on the rate of dissolution of human remains when added to a proportion of HCl was tested.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering an example of the taphonomic changes to expect and what lines of evidence persist in a container that could have held human remains in an acidic solution, in hopes of implementing policy change that would make these materials more difficult for criminals to access, therefore decreasing the potential for copycat crimes of this nature.

Due to the growing excitement and popularization of television crime shows, forensic scientists are now faced with the difficult task of analyzing copycat crime scenes, such as those portrayed on *CSI* or *Breaking Bad*. In particular, the disposal of human remains using common household acids has been mimicked by real criminals and led previous researchers to examine the effects of different acids on incomplete human remains. In general, previous studies have found that HCl is the most destructive agent when in contact with human remains, including fragments of femora and teeth.<sup>1-3</sup> HCl, commonly referred to as muriatic acid, is easily available for purchase at home improvement stores and sold in varying concentrations.

This present study expands on previous research by demonstrating the effect that HCl has on human remains in a controlled laboratory setting. Based on previous research with incomplete remains and experiences working on forensic cases, it was hypothesized that the HCl would not preferentially destroy some body parts over others (i.e., head and extremities over torso), and that the addition of estuarine water to HCl would not affect the rate of dissolution. This study tested these hypotheses with three experiments: the first involved an intact, donated human cadaver; the second used an intact pig carcass of similar size to the human; and the third examined the changes to eight donated human fingers. The remains were placed inside polypropylene plastic containers and submerged in a solution of 30% concentration of HCl (human and pig) or a proportion of HCl and estuarine water (fingers only). Quantitative variables included ambient and solution temperatures, pH, salinity, and cortical bone measurements. Qualitative variables included the presence of hair, nails and viscera, color of tissues (using Munsell color cards), and bone change (soft, eroded, pitted, gelatinous, amorphous, and total dissolution). These data were collected at hourly intervals (human=70 hours; pig=408 hours; human fingers=(208 hours) until the conclusion of the experiments after complete dissolution.

Results of the experiments revealed that the human and pig skulls were severely affected in just 13 hours after initial submersion in HCl. In addition, the head and extremities were almost completely dissolved in 70 hours (human) and approximately 87 hours (pig). The fingers in the third experiment took a total of five months to completely dissolve. Weight, solution temperature, and pH (<1) remained relatively consistent for all experiments, and portions of the torsos, including the viscera, persisted throughout the length of the human and pig experiments. Hair, finger and toe nails, and one dental restoration were recovered at the conclusion of the human experiment (Human Experiment 1=70 hours; Human Finger Experiment=3,720 hours). Teeth and nails were not recovered from the pig experiment=432 hours).

Contrary to the hypotheses of the researchers, these results indicated that the HCl could preferentially destroy certain body segments over others, and that pig remains follow a similar pattern of dissolution as that of human remains. Additionally, this study found that adding estuarine water to HCl reduces its dissolution efficiency while continuing to have an extremely acidic pH. These results provide a significant contribution to the current literature and offer an additional example of the taphonomic changes to expect and what lines of evidence persist in a container that could have held human remains in an acidic solution. Additionally, it is hoped this research assists legislators in implementing policy change that would make these materials more difficult for criminals to access, therefore decreasing the potential for copycat crimes of this nature.

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#### Forensic Anthropology, Decomposition, Hydrochloric Acid



### A65 Outdoor Crime Scene Reconstruction

*Dennis C. Dirkmaat, PhD\*, Mercyhurst University, Erie, PA 16546; Luis L. Cabo, MS, Mercyhurst University, Erie, PA 16546*

**Learning Overview:** After attending this presentation, attendees will have a clear understanding of new perspectives on the processing of outdoor crime scenes that focus on the roles of forensic archaeology, forensic osteology, and forensic taphonomy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by outlining how the disciplines of forensic archaeology and forensic taphonomy can have a significant impact on better reconstructions of the death event at outdoor crime scenes.

The discipline of forensic anthropology has changed significantly since it was first defined in the 1970s. Initially described as a laboratory-based, human osteology-centered discipline, the focus was almost exclusively on providing more accurate biological profile assessments that would aid efforts to identify unknown individuals found in a forensic setting. This study suggests that in the last few years forensic anthropology has evolved into two related but disparate sub-disciplines: (1) one focused on assessing biological profile at the time of death; and (2) another that focuses on the recovery and interpretation of human remains found in forensic contexts as one part of a medicolegal investigation. This sub-discipline involves scene-processing protocols utilizing forensic archaeological methods, practices, and principles; the consideration of remaining soft tissues, biological profile assessment, and interpretation of skeletal trauma in the laboratory; and, most importantly, the incorporation of forensic taphonomy into the construction of the most parsimonious, most evidence-based hypotheses of past events that transpired at the outdoor crime scene.

Law enforcement processing of the indoor scene is typically performed extremely well. However, a new approach to the processing of the outdoor crime scene is required. This new approach involves not only the thorough collection of the appropriate evidence, but an overarching set of guiding principles that focus on creating and testing viable and verifiable scientific-based hypotheses of what happened in the past at the scene. Forensic taphonomy fits the bill since it is particularly interested in the specifics of the removal/movement/alteration of the biological tissues and considerations of all relevant taphonomic agents from the time of emplacement until recovery. However, for law enforcement and medicolegal officials, reconstructing the death event (i.e., site formation), and not so much what has happened to the evidence since the site was formed, is what is important. From this perspective, the primary goal for processing a forensic scene is to provide a detailed sequence of events related to the death of the human victim: the crime scene reconstruction. At an outdoor scene, given different types of evidence and the important roles of forensic anthropology, forensic archaeology, and forensic taphonomy (not found in indoor scene recoveries), this presentation has labeled this activity: Outdoor Crime Scene Reconstruction (OCSR).

This newly described perspective and approach focuses on incidents surrounding the death event (the ephemeral, short-term event that oftentimes occurred months or years earlier) and includes three phases. Phase 1 requires the gathering of information from the outdoor forensic scene in order to reconstruct: (1) circumstances surrounding the death event; (2) the original location, position, and orientation of the body at the time of deposition on the scene; (3) what has happened to the remains since that time and whether humans have altered the scene; and (4) final Postmortem Interval (PMI) estimates. Location, documentation, and collection of evidence is best completed through forensic archaeology. During the recovery, multiple and varied hypotheses related to specific events of the past are constructed and tested. Phase 2 occurs in the laboratory. Remaining soft and hard tissue is analyzed for information critical to producing scientific estimates of PMI. Types and degree of impact of modifications to the biological tissues from various taphonomic agents (including humans) are noted. Final determination of specific bone, portion, and side for each skeletal element are made. Phase 3 of the OCSR approach involves the production of final hypotheses of past events. Scene information is melded with osteological information, and forensic taphonomy perspectives and paradigms of how to interpret the data are used to produce hypotheses of events surrounding the death event. These reconstructions are presented in the final report that is prepared as a courtroom-ready document.

#### **Outdoor Crime Scene Reconstruction, Forensic Taphonomy, Forensic Archaeology**





### A66 Use of an Alternate Light Source (ALS) to Locate Surface-Deposited Skeletal Remains

Tracy E. Brown\*, Herndon, VA 20170; Angi M. Christensen, PhD, FBI Laboratory, Quantico, VA 22135

**Learning Overview:** After attending this presentation, attendees will be aware of a new method for searching for skeletonized human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a method that can improve the quantity of bones and teeth recovered in cases of surface-deposited skeletal remains.

Searches for skeletal remains can be complicated by taphonomic processes that result in the disarticulation, fragmentation, and redistribution of remains. The consequence is often an incomplete recovery, with small bones, fragments, and teeth especially susceptible to being overlooked during a search. This study tests whether supplementing a traditional line search with a second search using an ALS, performed after dark, will result in the recovery of additional skeletal remains.

Forty-eight *Sus scrofa* bones and teeth, all measuring less than 4cm, were distributed and mapped within a ~21m x 11m search area. The search area had moderate surface debris, including leaves, grass, and sticks. Volunteer, inexperienced searchers were divided into 13 two-member teams and three search types. Four teams conducted a traditional daytime pedestrian line search only. Four teams performed a nighttime search only, using an ALS with a 450nm wavelength head and orange filter goggles. Five teams conducted a daytime line search followed by a nighttime ALS search. All finds were marked by the teams with pin flags. No time limit was imposed on the search (search teams determined when the search was complete), but the search time was recorded.

Teams conducting traditional daytime line searches located an average of 19 (40%) of the specimens, the ALS-only search teams located an average of 33 (69%) of the specimens, and the combined search resulted in an average of 37.4 (78%) of the specimens being located. When an ALS was used (in combination with a daytime search or alone), the number of specimens located was significantly greater compared to a traditional daytime line search only ( $p < 0.001$ ). Given that the searchers in this test were volunteers inexperienced in searching for skeletal remains, it is expected that those trained in evidence searches and/or identifying skeletal remains are likely to have higher recovery rates, but the relative differences between search team types is a significant finding. There was no significant difference in the time teams spent on daytime-only versus ALS-only searches ( $p=0.32$ ), and the combination of the two search types approximately doubled the total search time. For ALS and combined searches, there was a slight positive correlation between search time and specimens located ( $R^2=0.3997$  and  $0.0480$ , respectively); for daytime searches there was an inverse association between search time and specimens located ( $R^2=0.6747$ ).

For an ALS search to be effective in locating a bone or tooth, the skeletal specimen must be in the path of the light source and must retain fluorescent properties. This approach, therefore, would not be effective for locating buried remains or those obscured by significant surface debris, nor is it appropriate for locating severely burned remains or other remains significantly lacking in collagen. Searches conducted at night may involve environmental and security-related hazards, so time and safety considerations should be weighed against the benefits of locating additional remains.

The technique is easy to use, even by untrained personnel, and ALS devices are relatively affordable. Results of this study indicate that search approaches involving an ALS (either alone or in combination with a daytime line search) located significantly more remains than a daytime line search alone. It is recommended that traditional line searches be supplemented with an ALS search when possible, which is likely to increase the quantity of skeletal remains located.

#### Forensic Anthropology, Alternate Light Source (ALS), Search and Recovery



## A67 Evaluating the Use of Photogrammetry in the Excavation of Buried Human Remains

Kathleen Flor-Stagnato, MA\*, University of Tennessee, Knoxville, TN 37916-1013

**Learning Overview:** After attending this presentation, attendees will understand: (1) photogrammetric data collection and processing; (2) the limitations and strengths of photogrammetric data; and (3) the possibilities for human skeletal data collection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that photogrammetry can be a quick, inexpensive, and easily learned technique for creating spatially accurate and photorealistic renderings of burials or scenes.

Photogrammetry is defined by the American Society for Photogrammetry and Remote Sensing (ASPRS) as “the science or art of obtaining reliable measurements from photographs.”<sup>1</sup> The field of photogrammetry has changed rapidly with the public’s increasing access to tools and software. Increasingly popular, though not widely incorporated within forensic anthropology, is Structure from Motion photogrammetry, which creates digital 3D models from photographs.

Baier and Rando evaluated the use of photogrammetry in a simulated mass grave and stated that accurate cranial and post-cranial measurements would not be retrievable—post-excavation—from within the generated digital 3D model.<sup>2</sup> However, other studies have explored the use of photogrammetry for archaeological site recording and have found post-excavation measurements within the photogrammetric model not only possible, but accurate.<sup>3-8</sup>

This research evaluates photogrammetry in several ways: comparison between Geographic Information Systems (GIS) models and hand-mapping, the abilities of non-experts versus experts, and the ability to obtain accurate skeletal measurements from within the digital *in situ* model. The results demonstrate that photo collection time between skill levels did not vary significantly, and root mean squared error of the resulting models was also not significantly different between skill levels. Additionally, all data collected in the traditional excavation documentation process can be gathered or created from the photogrammetric models. Utilizing a photogrammetric workflow eliminates redundancies in the collection of data and can speed up work. Statistical analysis comparing the digitally taken skeletal measurements to the physically taken skeletal measurements revealed that accurate skeletal measurements are possible, which supports the findings of many other researchers and refutes Baier’s and Rando’s conclusions.<sup>2</sup> In order to obtain the best results from photogrammetric models, it is essential to follow a strict data collection protocol. If this is done, photogrammetry can be a useful tool to incorporate into the forensic anthropologist’s toolkit for documenting and analyzing a scene or excavation, which offers an accurate, fast, and inexpensive alternative to other current technologies and methods.

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### Photogrammetry, 3D, Buried Human Remains



### A68 Complexities in Transporting Human Remains Across International Borders

Denise To, PhD, DPAA-CIL, Joint Base Pearl Harbor-Hickam, HI 96853; Debra Prince Zinni, PhD\*, DPAA, Joint Base Pearl Harbor-Hickam, HI 96853

**Learning Overview:** After attending this presentation, attendees will better understand: (1) the complexities and regulations of conducting forensic anthropology in international jurisdictions; and (2) the importance of understanding local cultural and federal specificity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing examples of how regulatory legislation pertaining to transporting remains can vary greatly from country to country and by demonstrating how complex the process can be when physically transporting human remains across international borders.

With an increasing global trend of interaction between disparate societies, successful navigation of these complications can serve to improve international relations for all dedicated forensic anthropologists in the future.

Forensic anthropological work is conducted throughout the world and often at the request for international assistance. Organizations often call upon the forensic community to assist outside their respective countries when they encounter human remains as a result of armed conflicts, human rights violations and war crimes, past conflict accounting, terrorist events, natural disasters, and inadvertent exposure; however, a key concept in working internationally is having a solid foundation and firm understanding of foreign legislation pertaining to human remains. This presentation focuses on one of these aspects: transporting human remains across international borders.

The Defense POW/MIA Accounting Agency (DPAA) Laboratory specializes, in part, in recovering, collecting, analyzing, stabilizing, and transporting human remains in the international setting. DPAA investigation areas and recovery sites are located across the globe, and the movement of human remains oftentimes involves multiple international jurisdictions, as well as interlocking Department of Defense (DoD) agency responsibilities. In addition, due to the historical development of the accounting effort of missing United States servicemen, DPAA conducts forensic reviews with local federal scientists in numerous countries.

Regulatory statutes for processing archaeological remains is usually limited to within the country but can be complex as the policies of the local, state, and federal Historic Preservation Offices must successfully commingle with various First Peoples and Indigenous groups. However, comparatively speaking, the regulatory statutes for processing and transporting current-day death remains within and between countries is significantly more complex. Additionally, remains are oftentimes unilaterally offered to the DPAA from local nationals, and steps to ensure that local and indigenous remains are not being transported out of the country must be taken.

At the DPAA, the determination by any of their forensic archaeologists and/or forensic anthropologists that skeletal remains in their custody might be those of an American serviceman triggers a series of medicolegal steps that must be taken to transport those remains out of the country and bring them back to one of the DPAA laboratories. Considerations include: interest and involvement from local/state government, federal/host-nation officials, and United States State Department; local religious groups with vested interest; forensic and legal *laissez passer* requirements for human remains (or from local historic preservation and/or medicolegal offices), scientific review with local forensic scientists, biohazard and customs issues, death certificates, chain of custody documents; and even optics and media issues. The complexity of those steps varies greatly from country to country and even state to state. Some countries (e.g., Poland) place greater concern over the joint scientific review and less on optics and media, while other countries (e.g., Laos) are less concerned with permitting and paperwork and focus more on visibility and politics. Transportation of human remains through certain countries, such as Australia, requires emphasis on biohazard issues, while the transportation of human remains out of the Democratic People's Republic of Korea is at a complexity of the highest levels.

As a federal agency, the DPAA cannot afford any missteps in any part of these processes, as their actions in other countries have long-term implications. As forensic anthropologists, it is our responsibility to be diligent in navigating through these legal regulations as we assist and collaborate with forensic scientists beyond our borders.

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#### Forensic Anthropology, Transporting Remains, International



### A69 Qualifications for Forensic Anthropologists

Marin A. Pilloud, PhD, University of Nevada, Reno, Reno, NV 89557-0096; Nicholas V. Passalacqua, PhD\*, Western Carolina University, Cullowhee, NC 28723

**Learning Overview:** After attending this presentation, attendees will understand current attitudes toward various qualifications by forensic anthropology practitioners and will gain a clearer picture of where advances for the future should be focused.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by initiating the first steps for developing standards in the practice of forensic anthropology through a discussion of current and proposed qualifications for the discipline.

This presentation is based on an online survey hosted by Western Carolina University and approved by the Institutional Review Board of this institution as well as the University of Nevada, Reno. At the time of this writing, the survey had 258 respondents who, via self-reporting, had performed forensic anthropology casework or considered themselves qualified to do so. Note that not all respondents provided an answer to every question. In this overview of perceived and proposed qualifications in forensic anthropology, three main areas will be considered: education, training, and certification.

Education can be defined as the formal coursework from an accredited school, college, or university, while training can be defined as the formal, structured process of teaching and assessment at a laboratory or other non-educational institution.<sup>1</sup> Together, education and training form the basis for developing qualifications in a discipline.<sup>2</sup> Qualifications are typically demonstrated via certification or licensing by an accredited body. Presently, forensic anthropology lacks standards for qualifying practitioners in all three of these areas, and the reality is that many people are practicing forensic anthropology without appropriate qualifications.

In fact, when asked if they felt unqualified individuals were practicing forensic anthropology, 92% of respondents answered yes. When respondents were asked what would make an individual unqualified to practice forensic anthropology, the three most common responses were: a lack of training/experience (75%), followed by a lack of adequate education (32%), and unethical behavior (9%), respectively. Moreover, the clear majority of respondents (98%) were in favor of developing standards for education and training in forensic anthropology, and 75% supported developing an accreditation for forensic anthropology educational programs.

In terms of education, respondents had completed degrees ranging from a BA to a PhD. Courses taken during education also varied, with only three courses (human osteology, statistics, and archaeological theory) having been taken by more than 70% of all respondents during their education. When asked what knowledge areas should be required for the practice of forensic anthropology, 15 different areas were agreed upon by more than 70% of respondents, with human osteology being the primary subject area.

When asked what training the respondents had received, the most common answers were assisting with field-based (88%) or laboratory-based (79%) casework. When asked, "What do you think should be required for an individual to practice forensic anthropology?" more than 70% of respondents indicated, "Some period of work supervised by a certified forensic anthropologist" with various other choices, including certification or graduate degrees, falling below 70% agreement. However, in terms of training during education, only 44% of master's programs and 48% of doctoral programs attended by respondents had one or more anthropologists certified by the American Board of Forensic Anthropology (ABFA) as faculty.

Regarding certification, only 18% of respondents were certified by the ABFA. The responses toward certification by this organization were mixed (both strongly for and against); however, 94% of respondents were in favor of some form of certification for forensic anthropology.

The current push for scientific rigor and the publication of standards in the forensic sciences has presented an opportunity for forensic anthropologists to define qualifications for their discipline. These results demonstrate a clear need and desire for a standardization of qualifications within the field of forensic anthropology. Based on these results, efforts should initially be focused on outlining basic required coursework, which could potentially be overseen by an accrediting body (e.g., the Forensic Science Education Programs Accreditation Commission). Efforts should then move toward outlining training requirements to demonstrate individual competencies. Certifying bodies could then incorporate these standards in their application processes. In developing these qualification standards, focus should be on developing core competencies to practice forensic anthropology to best prepare practitioners for the discipline.

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2. Passalacqua N.V., Pilloud M.A. *Ethics and Professionalism in Forensic Anthropology*. San Diego, CA. Academic Press, 2018.

#### Qualifications, Education, Training



### A70 Harris County Forensic Anthropology Training Program

*Deborah C. Pinto, PhD\**, Harris County Institute of Forensic Science, Houston, TX 77054; *Michal L. Pierce, MS*, Harris County Institute of Forensic Sciences, Houston, TX 77054

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how a well-structured training program can increase the quality of future forensic anthropology practitioners. In addition, attendees will appreciate the need for support from an organization's top management as well as local and federal funding entities of such practical trainings in forensic anthropology, and more widely, forensic sciences.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing how to improve the quality of practice in forensic anthropology through rigorous training and mentorship.

This presentation provides a brief history of the forensic anthropology fellowship and internship program developed by the Harris County Institute of Forensic Sciences (HCIFS) as a possible template for other forensic anthropology training programs. The HCIFS serves a population of more than 4.5 million people and annually averages 4,000 autopsies, with approximately 350 forensic anthropology consults. As such, it provides a unique opportunity to facilitate practical training for forensic anthropologists. The Forensic Anthropology Division's (FAD's) training program has progressed through several iterations, with the most drastic changes occurring in 2015 when the FAD became accredited by the American National Standards Institute-American Society of Quality (ANSI-ASQ) National Accreditation Board (ANAB). The program encompasses training of interns, postdoctoral fellows, and newly hired staff.

Every summer, the HCIFS hosts up to a dozen interns who are mentored by professionals throughout the medical examiner office and crime laboratory. The FAD typically hosts one to two interns for approximately 12 weeks. These interns shadow each of the three staff anthropologists, as well as assist with case analyses, attend scenes, learn about laboratory quality assurance, and observe expert testimony; however, they do not co-author reports. During their internship, they receive periodic progress reports and evaluations from their mentors. Additionally, the interns are expected to conduct research or complete a special project and present their findings at a major conference.

Since 2010, the FAD has trained five postdoctoral fellows in casework under the FAD training program. This program was designed to mirror the in-house forensic pathology fellowship program, which is accredited by the Accreditation Council for Graduate Medical Education (ACGME). The fellows shadow staff anthropologists and eventually co-author cases during their fellowship year. They receive training in casework, including developing a biological profile, trauma analysis with special attention to pediatric trauma, assessment of pathological conditions, identification procedures, and scene recovery. In addition, they receive training in expert witness testimony and quality assurance. Like the interns, the FAD fellows conduct research or work on a special project and present their results at a major scientific conference.

To comply with ANAB accreditation standards, the FAD has developed a modular training manual, which allows customization of the training program to incoming interns, fellows, and newly hired staff. This type of program offers two tracks, allowing novices to receive more extensive training while seasoned practitioners can demonstrate competency in a more expedited manner. Once trainees successfully complete their training, they are provided with a memo of completion.

Exposing future forensic anthropologists to a diversity of casework within a quality assurance framework better equips them with the tools they need to adapt to any forensic environment. Each of the FAD fellows received training and/or conducted research through grant funding by programs under the National Institute of Justice or the Children's Justice Act. With their support, the FAD has successfully trained fellows who are currently board-certified or board-eligible forensic anthropologists and employed by various governmental and non-governmental organizations.

Due to the ever-increasing scrutiny of forensic practitioners, it serves forensic agencies well to ensure their anthropologists have a solid understanding and appreciation of quality assurance concepts, accreditation standards, and industry-accepted practices. Implementing a rigorous training program that incorporates these components can be labor and resource intensive; therefore, it requires support from top management, as well as federal and local funding entities. Bringing management on board can be facilitated by demonstrating the connection between well-rounded trainees and high-quality work.

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### Forensic Anthropology, Fellowship Training, Accreditation Program



### A71 The American Board of Forensic Anthropology (ABFA) Examination Turns 40: Historical Perspectives and Current Trends in Certification for Forensic Anthropology

*Eric J. Bartelink, PhD\*, California State University, Chico, Chico, CA 95929-0400; Donna C. Boyd, PhD, Radford University, Radford, VA 24142; Diane L. France, PhD, Human Identification Laboratory of Colorado, Fort Collins, CO 80524; James Pokines, PhD, Boston University School of Medicine, Boston, MA 02118; Debra Prince Zimi, PhD, DPAA, Joint Base Pearl Harbor-Hickam, HI 96853*

**Learning Overview:** The goal of this presentation is to highlight the history, development, and future of forensic anthropology board certification through the ABFA. Attendees will gain a better understanding of the role of the ABFA in certifying forensic anthropology practitioners and how the certification process has changed in recent years.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by providing a historical perspective on forensic anthropology board certification in the United States and Canada, as well as recent developments by the ABFA aimed at ensuring a transparent and fair examination process and the highest-quality practitioners.

The ABFA was founded in 1977, five years after the formation of the Physical Anthropology Section (now the Anthropology Section) through the American Academy of Forensic Science (AAFS). The ABFA was incorporated “as a non-profit organization to provide, in the public interest and the advancement of science, a program of certification in forensic anthropology” ([www.theABFA.org](http://www.theABFA.org)). Certification through the ABFA is considered the highest level of qualification in forensic anthropology. The ABFA is the only forensic anthropology certifying body in North America and is currently the only organization in the world that is accredited. In 2008, the ABFA received its accreditation through the Forensic Specialties Accreditation Board (FSAB), with re-accreditation cycles occurring every five years.

As of 2018, 119 individuals have been awarded Diplomate certificates. Of the 119 total Diplomates, 79 (66.4%) are considered in an active status. Inactive members represent Diplomates with retired status ( $n=16$ ), those who were decertified due to inactivity ( $n=6$ ), or those who are deceased ( $n=18$ ). From 1977–1978, certification was awarded to the first 22 applicants through a grandparenting process, representing 18.5% of the total awarded certificates. Beginning in 1979, applicants were required to sit for a written and laboratory practical examination. Successful passing of both sections with a score of 80% or higher is required to achieve Diplomate status. Growth of ABFA membership was initially slow, with 25 certificates being awarded in the late 1970s, 18 during the 1980s, and 16 during the 1990s. However, significant growth has occurred in the last two decades, with nearly one-third ( $n=37$ ) of the active membership added since 2010. These recently certified Diplomates represent more recent PhD’s who bring newer perspectives, skills, and experiences to the Board.

Although many aspects of the ABFA certification process have remained intact since the beginning, several significant changes have emerged in the past few years. The majority of changes reflect efforts to make the certification process more transparent, fair, and compliant with FSAB accreditation. During the application process, all submitted case reports must be redacted of any identifying information prior to submission. This allows the Board of Directors to evaluate case reports in the blind. For the board exam, each examinee selects a random identifier to also allow exams to be graded in the blind. For the written examination, the format was changed in 2011 from a series of essays to a multiple-choice format to facilitate more objective grading. Although exam questions have historically been solicited from all active Diplomates, recently, questions have been solicited from an Examination Question Committee composed of a representative number of Diplomates. Submitted questions must follow the provided multiple-choice template and are then screened by the validation committee and the vice-president. In addition, consistency in exam question structure and difficulty has been validated by an outside impartial entity to improve the structure and content of the exam. The test is generated from validated questions representative of key concepts in forensic anthropology, including the biological profile, determination of medicolegal significance, taphonomy and the postmortem interval, trauma analysis, assessment of pathological conditions and anomalies, legal procedures, personal identification, skeletal processing methods, scene recovery, analysis of commingled remains, specialized applications, laboratory procedures, statistics, and general skeletal biology. Examinees are now provided with a master list of key concepts and references as part of the study guide. The ABFA continues to improve its application and examination procedures to reflect new developments and to ensure the highest and fairest standards for forensic anthropology certification.

#### ABFA, Forensic Anthropology, Certification



### A72 Exploring Educational Needs Beyond Technical Competency: Laboratory Management, Testimony, and Vicarious Trauma

Jennifer C. Love, PhD\*, OCME, Washington, DC 20024

**Learning Overview:** After attending this presentation, attendees will understand the educational needs of forensic anthropology students beyond traditional technical competencies. This presentation will explore the skills needed for effective laboratory management, providing appropriate court testimony, and managing vicarious trauma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting important components of forensic anthropology education needed to shape the next generation of practitioners.

Since the landmark Supreme Court decisions of *Daubert v. Merrell Dow Pharmaceuticals, Inc.* and *Kuhmo Tire Company, Ltd. v. Carmichael* and the release of the 2009 National Academy of Science Report *Strengthening Forensic Sciences in the United States: A Path Forward*, the field of forensic anthropology has made great strides in validating and improving reliability of analytical methods.<sup>1-2</sup> Furthermore, with raised awareness of the importance of laboratory accreditation following international standards, the practice of competency and proficiency testing has taken root. In addition to these advancements, the field of forensic anthropology would be further strengthened by developing standardized training modules in areas beyond technical competencies, such as laboratory management, court testimony, and managing vicarious trauma.

Laboratory accreditation following international standards such as International Organization for Standardization (ISO) 17025 and ISO 17020 is the gold standard for forensic laboratory management and soon may become a requirement for laboratories receiving federal support, including grant funds. However, the language of international standards and the management requirements are not intuitive. Students should be introduced to appropriate laboratory management that meets international standards during their coursework and not solely through on-the-job training. Incorporating these competencies into forensic anthropology curricula would prepare graduates for professional requirements beyond technical skills, including non-technical skills required to work in an accredited laboratory as a member of an interprofessional team of experts.

Analysis of anthropologic evidence is the first step of case processing that often culminates in testimony. The requirements and limitations of forensic testimony are being defined as the United States Supreme Court is asked to rule on cases involving complex forensic analyses (*Melendez-Diaz v. Massachusetts*, *Bullcoming v. New Mexico*, and *Williams v. Illinois*).<sup>3-5</sup> Students must have a sound understanding of court proceedings, as well as the professional responsibilities, ethical obligations, and rights of expert witnesses. They must be prepared to answer appropriate questions with clear, concise answers that are palatable by the jury and to decline fielding questions outside of their expertise or role in the case and, therefore, best handled by other experts. Negotiating the witness stand is not a skill that should be learned on the witness stand, but rather learned in the classroom through targeted, outcome-focused training modules.

Vicarious trauma is emotional residue that practitioners experience from hearing trauma stories and witnessing pain, fear, and terror that trauma survivors have endured. Few anthropologists function solely in a role of a forensic scientist, receiving and analyzing specimens with no exposures to the individuals connected to the evidence. Most anthropologists, especially those employed by medical examiner offices, wear multiple hats, putting them in direct contact with family members of the recently deceased. This exposure places anthropologists at risk for vicarious trauma. Developing core competencies in the areas of professional development and personal growth that provide students with healthy coping mechanisms to respond to stress is needed.

Laboratory management, expert testimony, and navigating vicarious trauma are skills needed by forensic anthropologists that are equally as important as well-recognized technical competencies (e.g., estimating sex, ancestry, age, and stature) for performing at the level of a practicing professional. Forensic anthropology students must be introduced and become competent in these areas prior to serving as practitioners. The field of forensic anthropology will be greatly strengthened by developing and employing standard educational models, program curricula, and continuing professional education focused on developing these competencies.

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3. *Melendez-Diaz v. Massachusetts*, 557 U.S. 305 (2009).
4. *Bullcoming v. New Mexico*, 564, U.S. 647 (2011).
5. *Williams v. Illinois*, 567, U.S. 50 (2012).

#### Forensic Anthropology, Education, Core Competencies



### A73 Core Competencies and Entrustable Professional Activities: A Model for Forensic Anthropology Qualifications

Natalie R. Langley, PhD\*, Mayo Clinic School of Medicine, Scottsdale, AZ 85259; MariaTeresa A. Tersigni-Tarrant, PhD\*, Saint Louis University School of Medicine, St. Louis, MO 63104

**Learning Overview:** After attending this presentation, attendees will be able to discuss the potential for clearly delineated competencies to guide education, training, and certification in forensic anthropology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing a model for defining and assessing competencies of forensic anthropology trainees and practitioners.

A cascade of changes in forensic research, practice, and training has occurred since the landmark 1990s court rulings (e.g., *Daubert* and *Kumho*) and the 2009 National Academy of Sciences (NAS) Report, *Strengthening Forensic Sciences in the United States: A Path Forward*. As a result of work in this arena by the Department of Justice and National Institute of Standards and Technology, the Organization of Scientific Area Committees was created to replace the Scientific Working Group model for developing evidence-based standards and guidelines for forensic disciplines.

The forensic anthropology community has risen to the challenge of validating methods and improving reliability in our practice. The American Board of Forensic Anthropology has continually improved the certification process, and several forensic anthropology laboratories have undergone the rigorous process of accreditation. However, the discipline currently lacks uniform standards for education, training, and qualifications. In this environment of scientifically rigorous research and practice, the trickle-down implications of this absence of uniformity may be costly for our discipline.

A potential model for defining competencies to guide education, training, and certification is that of medical education: core competencies and Entrustable Professional Activities (EPAs). In 1999, the Accreditation Council for Graduate Medical Education (ACGME) selected six core competencies that captured the foundational skills every practicing physician should possess. These competencies have shaped educational programs and assessments of residents and undergraduate medical students. The ACGME is now laying the groundwork for using EPAs for resident competency assessment. EPAs are units of professional practice to be entrusted to an unsupervised trainee once he/she has attained sufficient competence in the task or responsibility. EPAs provide a means to translate the more abstract competencies into clinical practice. Whereas core competencies are descriptors of physicians, EPAs are descriptors of work—activities that characterize clinical and professional skills directly linked to the core competencies.

Competencies constitute a framework that describes the qualities and qualifications of professionals, and EPAs translate this framework into the world of practice. A competency-based model of education would ensure that trainees demonstrate a sufficient level of proficiency at the completion of the training. Furthermore, the competencies would provide uniform standards for assessment and certification of practitioners, as well as guide curriculum development and program accreditation. Competencies are outcomes-based, not process-based, and they evaluate the integration of knowledge, skills, and attitudes into practice.

This presentation outlines seven core competencies for forensic anthropology and will provide examples of EPAs for each of these competencies: (1) Knowledge for Practice: Knowledge of established and evolving practices in forensic anthropology and the application of this knowledge to casework; (2) Practice-Based Learning and Improvement: The ability to evaluate casework and research, to appraise and assimilate scientific evidence, and to continuously improve methods based on constant self-evaluation, reflection, and lifelong learning; (3) Interpersonal and Communication Skills: Interpersonal and communication skills that result in the effective exchange of information and collaboration with colleagues, students, medicolegal professionals, decedent families, and media representatives; (4) Professionalism: A commitment to maintaining professionalism and adhering to ethical principles in interactions with students, colleagues, medicolegal professionals, decedent families, and media representatives; (5) Systems-Based Practice: An awareness of and responsiveness to the larger context of the medicolegal system, as well as the ability to utilize system resources and collaborate with appropriate forensic subdisciplines to ensure optimal case outcomes; (6) Interprofessional Collaboration: The ability to engage in interprofessional collaborations in a manner that exemplifies the role and application of forensic anthropology in medicolegal settings; and (7) Professional Development and Personal Growth: The qualities and commitment required to sustain lifelong learning, professional development, and personal growth.

This presentation seeks to gather audience feedback and integrate input from various stakeholders regarding the potential for this competency-based model.

#### Competency, Certification, Professional Practice





### A74 Using Apatite Yield From Bone Sample Preparations for Quality Control in Stable Isotope Analysis Applications

Lesley A. Chesson, MS\*, PAE & DPAA Laboratory, Joint Base Pearl Harbor-Hickam, HI 96853; Gregory E. Berg, PhD, DPAA Identification Laboratory, Joint Base Pearl Harbor-Hickam, HI 96853-5530; Eric J. Bartelink, PhD, California State University, Chico, Chico, CA 95929-0400; Melanie M. Beasley, PhD, Knoxville, TN 37996-0720; Miranda Jans, PhD, SNA for DPAA, Joint Base Pearl Harbor-Hickam, HI 96853

**Learning Overview:** After attending this presentation, attendees will have a comprehensive understanding of the value of apatite yield for assessing quality control of bones prepared for isotopic analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a heretofore unutilized characteristic of bone bioapatite preparations (apatite yield) that can assist with evaluating sample quality prior to isotopic analysis.

Attendees will learn how calculated apatite yield could be used in addition to other quality assessment metrics (i.e., collagen yield, Carbon-to-Nitrogen (C/N), Infrared Splitting Factor (IR-SF), and Carbonate-to-Phosphate (C/P)) to screen bone samples. The importance of how sample quality control measures to ensure viable *in vivo* data are used when applied to forensic interpretations will be discussed.

Since the late 1970s, stable isotope ratios of bone have been a popular method for reconstructing various aspects of human history. In the mineral fraction (i.e., hydroxyapatite, bioapatite, or apatite), carbon isotope ratios of carbonate ions record diet while oxygen isotope ratios record geographical origin. The weight fraction of apatite in fresh bone has been measured at 57%.<sup>1</sup> Preparation of apatite for isotopic analysis requires the removal of collagen and non-collagenous proteins as well as “secondary” carbonates that may have been incorporated during diagenesis. Typically, bone powder is bleached using sodium hypochlorite or hydrogen peroxide to remove collagen, then treated with buffered or weak acetic acid to remove diagenetic carbonates.

Bone apatite preparation methods used in forensic investigations of unknown decedents—such as Jane and John Does, unidentified border crossers, and missing United States service personnel—have developed from the archaeological literature. The literature supplies several quality assessment metrics useful for screening bone samples. For apatite, these include the examination of changes in molecular structure using spectroscopy.<sup>2</sup> One method, Fourier Transform Infrared (FTIR) spectroscopy, is frequently used to assess apatite crystallinity through the measurement of an IR-SF. Additionally, the ratio of C/P in apatite is often measured via FTIR spectroscopy. While helpful for evaluating diagenesis, application of this semi-quantitative method requires a specialized spectrometer and substantial operator training.

This study investigates the utility of bone apatite yield as a quality assessment metric. Bone apatite and collagen fractions were prepared from 288 prehistoric (1,000 to 5,000 years B.P.) human samples from seven Central California sites and 191 “modern” (<100 years B.P.) human samples. The four accepted quality assessment metrics were measured: collagen yield, elemental composition as the atomic C/N ratio, IR-SF, and C/P. Apatite yield was additionally calculated and ranged from 21% to 68% across all samples.

Initial evaluation of apatite yield for quality control focused on prehistoric samples, which included bone in excellent to extremely poor condition. There was no correlation observed between apatite yield and either collagen yield or C/N; only weak correlations were observed between apatite yield and IR-SF ( $r^2=0.22$ ) or C/P ( $r^2=0.19$ ). Prehistoric samples were scored based on the traditional quality assessment metrics, with one point assigned for each unacceptable metric (defined as collagen yield <5%, C/N >3.5, IR-SF >3.5, and C/P <0.15). Samples were then categorized into one of four groups, with higher scores indicating poorer quality: 0, 1, 2, or 3-4 combined.

The “best” prehistoric samples, with no flagged quality metrics, had significantly lower mean apatite yield than samples with scores of 2 or higher (one-way Analysis of Variance (ANOVA) with Tukey’s multiple comparisons post-hoc test;  $F=15$ ,  $p < 0.0001$ ). Based on this, the “modern” samples—which are typical of bone used in isotope testing of unidentified decedents—were used to define expected apatite yield, with measurement error included, as 25%–60%.

This study demonstrates that apatite yield is useful in addition to, and potentially as a substitution for, other quality assessment metrics to screen bone samples prior to isotopic analysis. Unlike IR-SF or C/P measures, calculation of apatite yield requires no specialized instrumentation or training and is not determined from a semi-quantitative method. Routine calculation of apatite yield during bone sample preparation could additionally address needs related to necessary quality control monitoring within accredited forensic laboratories, including the demonstration of method repeatability and reproducibility. Given these initial results, it is proposed that bone sample preparations with apatite yield <25% or >60% should be considered unacceptable in forensic investigations.

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2. Beasley M.M., Bartelink E.J., Taylor L., and Miller R.M. Comparison of Transmission FTIR, ATR, and DRIFT Spectra: Implications for Assessment of Bone Bioapatite Diagenesis. *J Archaeol Sci*. 2014;46:16-22.

#### Isotopic Analysis, Bone Apatite, Quality Control (QC)

## A75 A Large-Scale Evaluation of Intraperson Isotopic Variation Within Human Bone Collagen and Bioapatite

Gregory E. Berg, PhD\*, DPAA Identification Laboratory, Joint Base Pearl Harbor-Hickam, HI 96853-5530; Eric J. Bartelink, PhD, California State University, Chico, Chico, CA 95929-0400; Jang Yuryang, PhD, National Defense Agency for KIA Recovery & ID, Seoul, SOUTH KOREA; Youngsoon Shin, MA, Republic of Korea MND Agency for KIA Recovery & ID, Seoul, SOUTH KOREA; Lesley A. Chesson, MS, PAE & DPAA Laboratory, Joint Base Pearl Harbor-Hickam, HI 96853

**Learning Overview:** After attending this presentation, attendees will have an in-depth understanding of the intraperson variation of Carbon (C) and Nitrogen (N) isotopic compositions of human bone collagen and C and Oxygen (O) isotopic compositions of human bone apatite. Forensic interpretative values for determining different individuals from isotope results will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving understanding of how isotope values of collagen and bioapatite vary within an individual.

Isotopic compositions of human tissues, such as bone or tooth enamel, are tied to diet or drinking water sources, thus allowing investigators to predict geographic origin of an unknown individual.<sup>1</sup> Some organizations, including the Defense POW/MIA Accounting Agency (DPAA), use isotope testing as a part of identifying unknown human remains. Isotope testing has multiple positive down-stream effects, such as limiting DNA testing and potentially separating commingled human remains.

Separating commingled human remains via isotope analysis is particularly important as mitochondrial DNA (mtDNA) can be shared between multiple individuals. For example, one DPAA commingled group has a skeletal Minimum Number of Individuals (MNI) of 23 individuals that all have similar mtDNA sequence information; to segregate them, additional full genome or autosomal DNA testing is necessary. Since autosomal DNA testing may fail half of the time for any one sample, other avenues are needed to solve this problem. Isotopic variation between individuals could potentially be used, but first we must determine what forensically meaningful intraperson variation is for each isotope and bone component.

The first widely cited “intra-individual” isotopic variation study was completed on rabbits and minks fed a monotonous diet.<sup>2</sup> This study concluded that the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of a single bone will be within 1‰ of the values obtained from other bones from the same individual, or from other individuals eating the same diet (to include humans). Since that 1983 publication, few other studies have explored this topic and only recently with human bone. Olsen et al. found that the maximum intraperson variation from four bones in six individuals was 0.6‰ for  $\delta^{13}\text{C}$  values and 1.6‰ for  $\delta^{15}\text{N}$  values, with a mean variation of 0.2‰ and 0.8‰, respectively. Fahy and colleagues<sup>4</sup> published on 10 skeletal elements from 10 individuals, finding the maximum intraperson variation to be 1.6‰ for  $\delta^{13}\text{C}$  values and 3.1‰ for  $\delta^{15}\text{N}$  values and mean variation to be 0.9‰ and 1.6‰, respectively.<sup>4</sup> Finally, a comprehensive literature review has not revealed any published intraperson variation data for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of human bone bioapatite.

This study isotopically analyzed both collagen and apatite from 5-6 long bone elements from 21 individuals for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values ( $n=112$  samples, 448 analyses). Samples were prepared at California State University, Chico; collagen was analyzed at the University of California, Davis, while apatite was analyzed at IsoForensics, Inc. This resulted in the largest known dataset of intraperson isotopic variation for human bone collagen and (per this study’s research) the only dataset for human bone apatite.

Initial results indicate that the maximum intraperson variation for collagen was 0.7‰ for  $\delta^{13}\text{C}$  values and 0.9‰ for  $\delta^{15}\text{N}$  values, with a mean variation of 0.2‰ and 0.6‰, respectively ( $SD=0.2\%$  each). For the apatite fraction, the variation was 1.1‰ for  $\delta^{13}\text{C}$  values and 1.2‰ for  $\delta^{18}\text{O}$  values, and the mean variation was 0.7‰ each ( $SD=0.2\%$  each). These results generally agree with the previously reported collagen data, though this study’s  $\delta^{15}\text{N}$  values have a smaller intra-individual range (possibly due to more consistent diets of forensic versus archaeological cases).

Using a two and three standard deviation from the mean model, it is proposed that any two bones that have differing collagen  $\delta^{13}\text{C}$  values greater than 0.75‰ are *probably* from different individuals, and those that have differing values greater than 0.95‰ are different individuals. Likewise,  $\delta^{15}\text{N}$  values greater than 1.0‰ are *probably* different, and greater than 1.2‰ are different. For apatite, the proposed values change slightly to 1.2‰ and 1.5‰ for  $\delta^{13}\text{C}$  values; for  $\delta^{18}\text{O}$  values,  $>1.2\%$ =*probably* different, while  $>1.4\%$ =*are* different. Following these parameters, sorting commingled human remains based on a triage of the isotope values of human remains can be undertaken.

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### Intraperson Isotopic Variation, Forensic Anthropology, Commingled Human Remains



## A76 Conservation of Isotope Ratios in Burned Bones of Pigs and Humans

Julianne J. Sarancha, MS\*, University of California, Davis, Davis, CA 95616; Gwyneth W. Gordon, PhD, School of Earth & Space Exploration, Tempe, AZ 85287-1404; Jelmer Eerkens, PhD, University of California, Davis, Davis, CA 95616; Christopher J. Hopkins, MS, Merck & Co, West Point, PA 19486; Eugenia Cunha, PhD, National Institute of Legal Medicine and Forensic, Coimbra, AB 3000-456, PORTUGAL; David Gonçalves, PhD, Research Centre for Anthropology and Health, Coimbra, PORTUGAL; Inês Santos, MSc, University of Coimbra, Coimbra 3000-456, PORTUGAL; Ana Vassalo, MSc, University of Coimbra, Coimbra 3000-456, PORTUGAL

**Learning Overview:** After attending this presentation, attendees will understand that isotope analysis can provide accurate inferences concerning diet and geographic origins in cases in which remains have been burned.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that isotope analysis can assist in identifying unknown remains that have been burned, but also constrain when the analysis may be less reliable.

Stable and radiogenic isotope analysis of unidentified human remains can assist forensic investigations by providing inferences about the individual's dietary and geographical life history. Understanding the effects of various postmortem processes on isotopic signatures is an important, but understudied, knowledge gap. Using two sample types and burn methods, this study was designed to replicate body disposal attempts by: (1) leaving surrounding tissues intact, (2) burning in an open flame, (3) introducing an accelerant, (4) comparing experimental samples to a control from the same individual, and (5) incorporating a control group from a single population to provide context for the magnitude of isotope differences.

This research hypothesized that isotope signatures of most isotope systems in modern bones are comparable before and after burning, focusing on commonly used isotope systems including carbon, nitrogen, oxygen, and radiogenic strontium, as well as lesser-studied isotope systems of sulfur and mass-dependent strontium. Fifteen racks of pig ribs, each rack providing one control and three to four experimental samples, originated from the same herd, ensuring the same isotope source for individuals. Leaving surrounding tissues intact, rib racks were sectioned and burned outdoors in a fire for a preassigned time (25min, 40min, or 55min) and accelerant treatment (none or diesel) while temperatures were recorded in 1min intervals (max. temp.=566°C). For human skeletal samples, two rib and two femur samples were taken from each of four dry skeletons. One rib and femur pair for each individual was kept as a control, and the other pair was burned in a furnace until temperatures reached 200°C, 400°C, 600°C, and 800°C. Isotope ratios were compared in unburned and burned samples from the same individual, which excluded inter-individual variation. Bone collagen, carbonate, and strontium were analyzed by Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS), Gas Bench-IRMS, and Multiple Collector-Inductively Coupled Plasma/Mass Spectrometry (MC-ICP/MS), respectively.

For the unburned controls (pigs), the range of each isotope system was  $\delta^{13}\text{C}_{\text{Collagen}}=2.54\text{‰}$ ,  $\delta^{15}\text{N}=2.62\text{‰}$ ,  $\delta^{34}\text{S}=4.0\text{‰}$ ,  $\delta^{13}\text{C}_{\text{Carbonate}}=2.16\text{‰}$ ,  $\delta^{18}\text{O}=8.64\text{‰}$ ,  $^{87}\text{Sr}/^{86}\text{Sr}=0.00097$ , and  $\delta^{88/86}\text{Sr}=0.28\text{‰}$ . For the pigs, the largest standard deviation ( $1\sigma$ ) seen within an individual, including burnt samples, for each isotope system was  $\delta^{13}\text{C}_{\text{Collagen}}=0.24\text{‰}$ ,  $\delta^{15}\text{N}=0.22\text{‰}$ ,  $\delta^{34}\text{S}=2.4\text{‰}$ ,  $\delta^{13}\text{C}_{\text{Carbonate}}=0.41\text{‰}$ ,  $\delta^{18}\text{O}=2.85\text{‰}$ ,  $^{87}\text{Sr}/^{86}\text{Sr}=0.00011$ , and  $\delta^{88/86}\text{Sr}=0.16\text{‰}$ . For the humans, the largest standard deviation ( $1\sigma$ ) seen within an individual for each isotope system was  $\delta^{13}\text{C}_{\text{Collagen}}=0.14\text{‰}$ ,  $\delta^{15}\text{N}=0.18\text{‰}$ ,  $\delta^{34}\text{S}=0.9\text{‰}$ ,  $\delta^{13}\text{C}_{\text{Carbonate}}=1.05\text{‰}$ ,  $\delta^{18}\text{O}=7.92\text{‰}$ ,  $^{87}\text{Sr}/^{86}\text{Sr}=0.00011$ , and  $\delta^{88/86}\text{Sr}=0.06\text{‰}$ . Overall, the variation for each isotope system between burnt and control samples was less than the range seen in the herd of pigs, except for  $\delta^{13}\text{C}_{\text{Carbonate}}$  and  $\delta^{18}\text{O}$  in human samples, which showed a decrease at 800°C and 400°C, respectively.

In cases such as body disposal attempts within the parameters explored in this study, it is likely that isotopic signatures will provide accurate dietary and geographical history inferences from bone, especially if surrounding tissues remain intact post-burn. Extreme burn cases, such as cremation or prolonged furnace heating of skeletal components, will likely alter the collagen and carbonate constituents.

### Isotope Analysis, Burned Bones, Forensic Geochemistry



### A77 The Role of Stable Isotope Analysis in Forensic Cases From Flagstaff, Arizona

*Taylor Lambrigger\*, Gilbert, AZ 85297*

**Learning Overview:** After attending this presentation, attendees will better understand the viability of stable isotope analysis in a forensics context, specifically assessing its potential to aid in forensic identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illuminating whether stable isotope analysis has the potential to be used in a forensics context in assisting with identification.

Coconino County, AZ, has a unique forensic profile. Home to a major state university that draws in students from a plethora of other states and countries, the Grand Canyon, which attracts millions of international and national visitors, as well as the Navajo and Hopi Native American reservations, the county handles numerous deaths each year of individuals from varying backgrounds. Those remains obtained by the Coconino County Medical Examiner's Office are often identifiable; however, those that are unable to be forensically identified are kept in storage and constantly reassessed for identification. Historically, analysis of stable isotopes has been a useful tool in various fields, including archaeology, to gain insight into the geological and biological information for both human and faunal remains. Because of the pertinent data regarding migration and diet that can be obtained from stable isotope studies, analysis of various stable isotopes in unidentifiable human remains allows for the building of biological backgrounds that can potentially assist in forensic identification.

Twenty-one femoral and dental samples from unidentifiable human remains were obtained from the Coconino County Medical Examiner's Office and transferred to Northern Arizona University's Paleodiet Lab. Teeth, which form in childhood and adolescence, allow for isotopic studies of the beginning of life, and, due to the remodeling of bones in the body, the isotopic levels from the last decade of life can be researched. Eleven individuals were studied, with six having a paired dental and femoral sample, allowing for the assessment of isotopic values throughout the life course. Additionally, the processing of remains allowed for the verification of provenience information cataloged by the Medical Examiner's Office, including estimated age and sex. Each sample was processed, followed by collagen and carbonate analysis for each femoral sample and carbonate analysis for dental samples. Furthermore, 11 samples were prepared for strontium and lead analysis. Upon obtaining the data from analysis, strontium and oxygen isotope levels were compared with recently developed isoscapes for both the United States and various other states in Western Europe to assess patterns of migration. Trends in carbonate and collagen data were also measured against known literature for dietary patterns of different regional populations in the United States.

The importance of this study's results is multifaceted. Primarily, a check on the accuracy of provenience data collected by the Coconino County Medical Examiner's Office was conducted, updating the records held for each individual. Isotopically, information on dietary patterns and geographic migrations were obtained. Results consistent with dietary patterns of individuals from the Navajo reservation were seen, as well as possible consistencies with Western European diets. Additionally, analysis of regional water intake potentially aided in geographic data obtained for individuals. Furthermore, valuable data regarding the relevance of stable isotope studies in contemporary human studies was seen, as trends in breastfeeding and weaning that are consistent with American trends were confirmed isotopically. With the increase of globalization potentially undermining isotopic studies on contemporary populations, this study demonstrates that there is information that can be gathered that can aid in forensic identifications.

#### **Isotope, Unidentifiable, Identification**



### A78 Experimental Lacustrine Taphonomy: Decompositional Changes in Freshwater Lake-Submerged Skeletal Remains

Vienna C. Lam, MA\*, Simon Fraser University, Burnaby, BC V5A 1S6, CANADA; Lynne S. Bell, PhD, Simon Fraser University, Burnaby, BC V5A 1S6, CANADA

**Learning Overview:** After attending this presentation, attendees will better understand how *Ovis aries* (domestic sheep) bones will change at the macroscopic and microscopic level when submerged in freshwater lakes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating what aquatic taphonomic signifiers can be expected of freshwater submergence of cortical bone discs in the Pacific Northwest and how findings can be used to compare with other submergence contexts, ecological zones, and geographic regions.

Although submergence studies have identified changes to skeletal remains that help distinguish aquatic decompositional environments from those involving terrestrial exposure, few studies have distinguished whether freshwater contexts would result in different diagenetic changes than that of marine water. Existing literature in this area has also inconsistently reported the presence of sediment-induced abrasion, disarticulation, dissolution, encrustation, fragmentation, spatial orientation, bioerosional tunneling, mechanical weathering, fluvial erosion, and scavenging marks, which suggests that taphonomic signifiers may be bound to ecological zones. Being able to identify taphonomic signifiers associated with freshwater lakes is also of forensic interest because it may help detect postmortem secondary movement and elapsed time since entering the water. The goal of this study was to create a baseline of decompositional changes in freshwater submerged skeletal remains and to explore whether these changes can be attributed to elapsed time, seasonality, and/or specific depositional environments across a freshwater lake.

This aquatic field experiment involved deploying sectioned sub-adult *Ovis aries* (domestic sheep) femoral bone discs ( $n=130$ ) across ten sites across Marion Lake, BC, Canada, to study the effects of freshwater submergence on cortical bone preservation. Control samples were secured in the Centre for Forensic Research, Simon Fraser University, Canada. Experimental specimens were recovered consecutively over a 16-month period from 2016–2017 and analyzed macroscopically for structural (artifact, abrasion, cracking, bioerosion) and color change using a Zeiss Stemi microscope. Atmospheric, lake surface, and core temperature were also monitored, along with precipitation, water pH, cage movement, and elemental profiling of silt composition using Focused Ion Beam/ Scanning Electron Microscopy (FIB/SEM) and Energy Dispersive Analysis X-Ray (EDAX).

Results revealed a significant relationship between several taphonomic signifiers and the location of submergence and elapsed time of submergence. Periosteal abrasion was found to be significantly related to the length of the submergence period. This suggests that the longer the sample stayed underwater, the more abrasive wear took place due to natural hydrodynamic characteristics of a mountainous coastal lake. Where samples were placed across Marion Lake had no effect on whether cracking would occur. Instead, the length of time a sample was submerged was yet again the most important factor in determining whether cracking on both the periosteum and transverse block face would occur. It was hypothesized that cracking would follow weaker boundaries of existing lamellar alignment, but findings reveal that cracks were tracking the periosteal surface inward, as well as radiating outward from cutting cones. Darkening of bone tissue from white to brown was found, along with blue and green staining on the surface of samples. The location of the cages was linked to the appearance of periosteal abrasion and encrustation, and distinctive damage to the periosteal surface was observed. Saw marks and bone lipping in submerged samples were also obscured or obliterated over time. This loss of pre-deployment artifacts suggests that intentional human-induced disarticulation of bones might be obscured over time. None of the control samples maintained in the laboratory exhibited any of the changes observed in experimentally submerged samples. The results of this experimental study on lake-submerged bone documented taphonomic changes that take place when submerged in freshwater lakes within the Pacific Coastal Western Hemlock Zone.

#### Taphonomy, Forensic Anthropology, Freshwater Decomposition



### A79 Modeling the Fluvial Transport of Human Remains in the Sacramento River of California

Colleen F. Milligan, PhD\*, California State University, Chico, Chico, CA 95929-0400; Eric J. Bartelink, PhD, California State University, Chico, Chico, CA 95929-0400

**Learning Overview:** After attending this presentation, attendees will better understand the relationship between water discharge rates and the transport of human remains in riverine systems. The primary goal of this presentation is to discuss the development of a predictive fluvial transport model to aid in the search and recovery of human remains in the Sacramento River of California.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the use of fluvial transport rates of rescue manikins to predict search parameters for human bodies from riverine systems. This presentation will discuss the variables that contribute to differences in transport rates, such as postmortem interval, flow rates, river dimensions and curvature, riverbed substrate, and the prevalence of snags or obstacles.

The fluvial transport of human remains is a topic of interest in paleoanthropology, bioarchaeology, and forensic anthropology. In the context of taphonomy, fluvial transport focuses on the potential of a riverine system or body of water to physically move human remains from one location to another. Missing person's cases involving riverine systems pose major challenges to law enforcement given that human remains may be moved a long distance from their initial point of entry, especially under high flow rate conditions. However, very little actualistic research has been conducted to model the movement of human bodies in riverine systems, such as the Sacramento River.

The Sacramento River is California's largest fluvial system. Flow rates are regulated by Shasta Dam at the river's source and are influenced by the numerous tributaries that feed into the river, as well as by the physical characteristics of the river. The highest flow rates occur during winter storm events and the lowest during the late fall, after irrigation season has ended. Each year, the river claims more than two dozen victims, most commonly due to accidental drowning. A much smaller number of bodies recovered from the river represent suicides, homicides, or have an undetermined manner of death.

Although the Sacramento River spans eight counties, the project area includes the five counties from the southern half of the Sacramento River. This research adapts an existing hydraulic model (Hydrologic Engineering Center's River Analysis System (HEC-RAS)) to generate a predictive model of fluvial transport rates of victims who entered the river with known dates and locations under low, medium, and high flow rate conditions. The HEC-RAS was originally designed for flood control management, but also has capabilities to simulate advective and dispersive transport of water quality constituents. The HEC-RAS model treats a human body as a "pollutant" and can be used to predict distance from the source given a number of parameters, such as flow rate conditions and river channel properties. The model is calibrated using two sources of information: historical case data on river victims collected from sheriff-coroner's offices and data generated from rescue manikins placed in the river under different flow rate conditions.

Historical data was collected on 150 river victim cases from three counties in the project area (spanning 1972–2012), 62 (41.3%) of which had known postmortem interval and transport distance data. This initial stage of data collection demonstrated that most victims are male, and most deaths are accidental. A moderate relationship between postmortem interval and transport distance was found, but postmortem interval only explained a small part of the variation in transport distance. To supplement the historical data, this study used two types of rescue manikins to simulate the body positions in water of "floaters" and "sinkers." Controlled releases of the floating manikins were conducted under low, moderate, and high flow rate conditions from six locations along the Sacramento River. The results of these simulations have shown that the river curvature and flow rate play a large role in the route of travel a body takes, with repeated tests in the same locations showing similarity in the path the body travels. The project has also used sinker manikins to study the transport of human remains along the riverbed. Data collected at four locations, including a concrete test flume, has shown that there is a significant relationship between type of riverbed substrate and the flow rate (measured in feet/second) and transport distances of human remains.

*This research was funded by a National Institute of Justice (NIJ) grant.*

#### Forensic Anthropology, Taphonomy, Fluvial Transport



### A80 Introducing Forensic Investigation Research Station (FIRS-TB40): Scavenger Succession and Progression at a High-Altitude Site in Colorado

Christiane Baigent, MSc\*, Forensic Investigation Research Station, Grand Junction, CO 81501; Melissa A. Connor, PhD, Colorado Mesa University, Grand Junction, CO 81501-3122; Gretchen R. Dabbs, PhD, Southern Illinois University, Carbondale, IL 62901

**Learning Overview:** After attending this presentation, attendees will better understand how scavenger guild interaction affects decomposition at high altitude.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new human decomposition research site, and the taphonomy of a high-altitude ecoregion, the variables of which are not well understood in a forensic context.

Studies of decomposition at high-altitude are limited and report distinct changes in decomposition with increases in altitude.<sup>1,2</sup> Colorado's Rocky Mountain region bisects the state from north to south and is classified as a fully humid, snowy climate interdigitated by polar regions that reach elevations greater than 14,000 feet Above Mean Sea Level (AMSL). High altitude is correlated with an increased incidence in suicide, sudden-onset high mortality edemas, and a suite of physiological changes associated with the composition of partial pressure of atmospheric gasses. Additionally, remote expanses of wilderness provide ideal locations for clandestine body disposal. These variables yield a high incidence of remote, complex, outdoor human decomposition scenes characterized by prolonged exposure, advanced decomposition, and animal scavenging.

With the support of the Park County Coroner and Commissioners, the Forensic Investigation Research Station at Colorado Mesa University acquired 40-acres of land in the Rocky Mountain Region of Colorado (FIRS-TB40). FIRS-TB40 is 9,500 feet AMSL in the South Park region of the central Rockies. The site is bisected north to south by a steep, rocky, moraine-like ridge covered in short prairie grass and stunted juniper.

Four pigs were placed in the summer of 2018 in anticipation of placing a human cohort in 2019. Of primary interest was the succession and progression of local scavenger guilds. Four carrions (two exposed/two caged) were placed in a longitudinal transect across a 45° slope, each 30 meters apart. Each sample was monitored by a game camera programmed to collect both time lapse photographs and respond to motion in the environment. Carrion were visited three times weekly for a period of eight weeks, photographed, and decomposition documented using the total body score model.<sup>3</sup> When scavenging occurred, a site search was performed in ordered, longitudinal transects to locate scattered skeletal material.

Arthropod and avian communities constituted the first wave of scavenger succession; activity in both communities began almost immediately after deposition. Avian activity was limited to non-caged carrion, despite aerial access to the cages, while arthropods were active at all carrion sites. Turkey vultures (*Cathartes aura*) were the dominant species early in succession, followed by ravens (*Corvus corax*). High inter-species tolerance was demonstrated by co-scavenging, but vultures dominated in population size and tissue exploitation in the initial phase. Scavenging began in rectal tissue and progressed to the retro-otic region, the length of the thorax, and the limbs, in both uncaged carrion. Evidence for avian scavenging included distinct claw marks in superficial perianal tissue and along the length of the thorax, bore holes in the cranial region, shallow, linear beak striae in superficial tissue, and ribboning of connective tissue. Insects preferentially oviposited at the ground/body interface. Two pigs rolled down the slope following bloat. Reorientation of the carrion exposed previously sheltered larvae to the environment, delaying or arresting the development of maggot masses.

Coyotes (*Canis latrans*) arrived seven days postmortem and constituted the second wave of scavengers. While capable of removing greater amounts of tissue in a single visit, reduction was largely limited to circumscribed areas and progressed across 21 days. Scavenging commenced at the hind limbs, followed by the head, the front limbs, and the thorax. Coyotes moved scavenged carrion across and downslope—behavior anticipated based on extant knowledge of slope gravity and the law of least energy expenditure. However, osseous evidence indicated canids carried the carrion up the steep slope to the hill crest to consume, behavior that runs counter to widely published patterns. American black bears (*Ursus americanus*) arrived 21 days postmortem and constituted the final wave of the succession. Bears were anticipated to act as apex scavengers. However, while observed in direct proximity to all carrion, they did not choose to scavenge.

Scavenger behavior is dictated by reflexive patterns or cooperation and competition that are regionally distinct and have the potential to impact human remains recovery. This study demonstrates the necessity of considering scavenger behavior in concert to effectively understand behavior that diverges from the norm and regional patterns of taphonomic change.

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#### High Altitude, Scavenger Succession, Human Decomposition



**A81 WITHDRAWN**





### A82 Virtual Forensic Anthropology: The Accuracy of Osteometry on 3D Virtual Models of the Os Coxa Derived From Clinical Computer Tomography (CT) Scans

*Kerri L. Colman, MSc, Amsterdam Medical Centre, Amsterdam, North Holland 1077 ZH, NETHERLANDS; Hans H. De Boer, MD, PhD\*, Amsterdam University Medical Center - AMC, Amsterdam, Zuid-Holland 1105 AZ, NETHERLANDS; Johannes G.G. Dobbe, PhD, Academic Medical Center, Amsterdam 1100 AZ, NETHERLANDS; Niels Liberton, VUmc, Amsterdam, Noord-Holland 1081HV, NETHERLANDS; Kyra E. Stull, PhD, University of Nevada, Reno, Reno, NV 89557; Maureen van Eijnatten, PhD, Amsterdam UMC, Amsterdam, Noord-Holland 1081 HZ, NETHERLANDS; Johannes G. Streekstra, PhD, Academic Medical Center, Amsterdam 1100 AZ, NETHERLANDS; Roelof-Jan G. Oostra, PhD, Academic Medical Center, Amsterdam 1100 AZ, NETHERLANDS; Rick R. Van Rijn, PhD, Amsterdam UMC, Amsterdam 3544MT, NETHERLANDS; Lida A.E. Van der Merwe, PhD, Academic Medical Center, Amsterdam 1100 AZ, NETHERLANDS*

**Learning Overview:** This study seeks to address two important issues in “virtual” forensic anthropology: (1) the currently insufficient knowledge regarding the accuracy of 3D virtual reconstructions from clinical CT scans; and (2) the effect of 3D reconstructions on landmark recognition, which is pivotal for reliable measurements.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the accuracy of virtual models derived from clinical CT scans and how this might impact “virtual” forensic anthropology.

**Background:** Clinical radiological data (i.e., CT scans) are a promising source for contemporary population-specific data to test or develop forensic anthropological methods. However, the feasibility of this approach is unclear since: (1) the accuracy of 3D virtual reconstructions from clinical CT scans is insufficiently known; and (2) the effect of 3D reconstructions on landmark recognition (pivotal for reliable measurements) is largely unknown. This study seeks to address both these issues.

**Materials and Methods:** Twenty-seven (13 males, 14 females) fully intact cadavers from the body donation program of the Amsterdam UMC, University of Amsterdam, Department of Medical Biology, section Clinical Anatomy and Embryology were CT scanned using a patient scanning protocol (referred to as the “Fleshed CT”). Subsequently, the bodies were processed to obtain the dry os coxae and CT scanned again using the same scanning protocol (referred to as “Dry CT”). Both scan sets were used to render 3D virtual skeletal elements (“Fleshed CT virtual models” and “Dry CT virtual models”). Then, an Artec Spider 3D optical scanner was used to produce 3D models of a subset of ten dry os coxae from the sample. Given its minimal error (accuracy <0.05mm) and high resolution (~0.1mm) the optical scanner was used as the “gold standard.”

The accuracy of the Fleshed and Dry CT models was calculated by assessing the deviation (in mm) to the gold standard. This was done for the overall os coxae (left and right combined) and for Regions Of Interest (ROI) representing selected Landmarks (LMs). To compare the error associated with landmark recognition, the intra- and inter-observer error (Technical Error of Measurement (TEM) and %TEM) of nine traditional Inter-Landmark Distances (ILD) was measured on all dry os coxae and the 3D virtual models (Fleshed CT and Dry CT).

**Results:** Fleshed CT virtual models were found to be 0.64mm-0.88mm larger than the gold standard (deviations ranging from -4.99mm to 5.00mm). Dry CT virtual models were 0.36mm-0.45mm larger than the gold standard (deviations ranging from -0.27mm to 2.86mm). The accuracies at the various ROIs were variable and larger for Fleshed CT virtual models than for Dry CT virtual models.

For all three sets of virtual models, intra- and inter-observer error was in the generally acceptable range (TEM<2mm, %TEM <2%) for all ILDs, except for three ILDs all of which included the landmark located on the most inferior point on the ischial tuberosity.

**Discussion and Conclusions:** These results demonstrate that virtual models developed from clinical (i.e., “fleshed”) CT scans are larger than the scanned skeletal element. The same holds for virtual models developed from “dry bone” CT scans, although the difference is less pronounced. In both groups, this overestimation in size is on average <1mm for the overall os coxae; however, this difference in size between the modalities (Fleshed CT versus the gold standard and Dry CT versus the gold standard) has practical implications when constructing ILDs from the ROIs associated with the various LMs. Corresponding ILDs taken from ROIs with large (>1mm) variability on different modalities may result in ILDs greater than the generally accepted 2mm error in forensic anthropology. The majority of morphological LMs were consistently identified, and the use of 3D models thus apparently doesn’t affect the recognition of osteometric landmarks substantially.

Based on these results, it is argued that clinical CT data should only be used as a source for forensic anthropological reference data when the (sometimes considerable) shape and size differences between the virtual models and the dry skeletal elements are considered. Furthermore, osteometric methods for the estimation of sex, age, stature, and ancestry derived from dry skeletal elements cannot be readily applied to 3D reconstructions from clinical CT scans (and vice versa).

#### Virtual Forensic Anthropology, Metric Sex Estimation, Accuracy

### A83 Virtual Anthropology: The Interchangeability of Morphological Sex Estimation Methods on 3D Models of the Pelvis and Their Dry Skeletal Counterpart

Kerri L. Colman, MSc, Amsterdam Medical Centre, Amsterdam, North Holland 1077 ZH, NETHERLANDS; Lida A.E. Van der Merwe, PhD, Academic Medical Center, Amsterdam 1100 AZ, NETHERLANDS; Kyra E. Stull, PhD, University of Nevada, Reno, Reno, NV 89557; Aubrey Van Het Reve, BSc, AMC, Amsterdam, Noord-Holland 1100 AZ, NETHERLANDS; Hannah E.M. Crijns, BSc, AMC, Amsterdam, North-Holland 1100 AZ, NETHERLANDS; Johannes G.G. Dobbe, PhD, Academic Medical Center, Amsterdam 1100 AZ, NETHERLANDS; Johannes G. Streekstra, PhD, Academic Medical Center, Amsterdam 1100 AZ, NETHERLANDS; Rick R. Van Rijn, PhD, Amsterdam UMC, Amsterdam 3544MT, NETHERLANDS; Roelof-Jan G. Oostra, PhD, Academic Medical Center, Amsterdam 1100 AZ, NETHERLANDS; Hans H. De Boer, MD, PhD\*, Amsterdam University Medical Center - AMC, Amsterdam, Zuid-Holland 1105 AZ, NETHERLANDS

**Learning Overview:** The goal of this presentation is to inform attendees as to whether commonly used pelvic morphological methods (e.g., those incorporated in the Klales and the 1980 Workshop of European Anthropologists (WEA) morphological sex estimation methods) can be accurately scored on reconstructed virtual 3D pelvic bone elements.<sup>1,2</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees on the interchangeability of morphological sex estimation methods from virtual bone models to dry skeletal elements. This is a pivotal prerequisite for the use of Computed Tomography (CT) scans as a reference for forensic anthropological case work.

**Background:** For forensic anthropological sex estimation methods to be admissible in the court of law, they need to agree with the *Daubert* standards, and thus derived from or tested on large and representative skeletal populations. However, most European countries lack representative collections and are thus unable to develop or test sex estimation methods. The large amount of clinical CT data in hospitals could serve as a basis for a 3D virtual skeletal database and thus provide a solution, but it is uncertain if the often-subtle features associated with morphological sex estimation can be scored accurately from virtual 3D skeletal elements.

The current study aims to determine whether commonly used pelvic morphological methods (e.g., those incorporated in the Klales and WEA morphological sex estimation methods) that utilize ordinal scales can be accurately scored on reconstructed virtual 3D pelvic bone elements.<sup>1,2</sup>

**Materials and Methods:** Twenty-seven (13 males, 14 females) randomly selected and fully intact cadavers from the body donation program of the Amsterdam UMC, University of Amsterdam, Department of Medical Biology, section Clinical Anatomy and Embryology were CT scanned using a standard patient scanning protocol. The scan data were used to produce 3D virtual pelvic elements, and the os coxae were segmented with an in-house research software. Following CT scanning, the bodies were processed to obtain the corresponding dry skeletal elements.

Three Phenice traits expanded to an ordinal scale by Klales and five selected WEA traits were scored on both the virtual bone models and the dry bone counterparts.<sup>1,2</sup> Intra- and inter-observer agreement, and the agreement between the virtual bone models and their dry skeletal counterpart, was calculated using Cohen's weighted Kappa (K).

**Results:** For all Klales traits investigated, Kappa values showed substantial to almost perfect agreement for intra- and inter-observer agreement, respectively, on both the virtual bone models (K=0.78-0.84 and 0.62-0.72) and the dry bones (K=0.78-0.89 and 0.61-0.77), when scored in isolation.<sup>1</sup> When comparing corresponding virtual- to dry bone pelvic elements, all three traits show substantial to almost perfect agreement (K=0.74-0.82).

Only two of the five WEA traits (pre-auricular sulcus and pubic angle) showed substantial to almost perfect agreement for intra- and inter-observer agreement, respectively, on both the virtual bone models (K=0.71-0.80 and 0.65-0.82) and the dry bones (K=0.80-0.93 and 0.76-0.88), when scored in isolation.<sup>2</sup> The remaining three traits showed poorer results for inter-observer agreement, with the Greater sciatic notch and the Arc compose resulting in fair to moderate agreement (K=0.51 and K=0.54, respectively), while the Ischial body showed less than chance to fair agreement (K=0.054-0.33).

When comparing corresponding virtual to dry bone pelvic elements, only the Greater sciatic notch, sub-pubic angle, and Arc compose show substantial to almost perfect agreement (K=0.68-0.85).

**Discussion and Conclusion:** These results suggest that the Klales morphological sex estimation technique can be used interchangeably between virtual bone models and dry skeletal elements.<sup>1</sup> However, the same cannot be said for the five selected WEA pelvic traits addressed in this study.<sup>2</sup> Despite the scores for the Greater sciatic notch, sub-pubic angle, and Arc compose accurately corresponding on both the virtual bone models and the dry skeletal elements, due to the lack of inter-observer agreement, only the sub-pubic angle can be used interchangeably. This study shows the importance of testing the differences between the virtual bone models and the dry skeletal elements before methods are applied interchangeably.

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#### Virtual Forensic Anthropology, Morphological Sex Estimation, Accuracy



### A84 Perceptions and Cognitive Bias in Decomposition Scoring Methods in Forensic Anthropology

Kelly Sauerwein, PhD\*, University of Tennessee, Knoxville, TN 37996; Xiaojuan Zhu, University of Tennessee, Knoxville, TN 37996; Dawnie W. Steadman, PhD, University of Tennessee, Knoxville, TN 37996

**Learning Overview:** After attending this presentation, attendees will better understand how cognitive biases may impact the interpretation of human remains and the decision-making process involved when using the Total Body Score (TBS) method.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the gaps in our understanding of bias in an oft-utilized method that is underrepresented in previous studies of bias in forensic anthropology.

Since *Daubert* and the 2009 National Academy of Sciences (NAS) Report, there has been increasing recognition within the forensic science community that human examiners are prone to biases that may affect the accuracy and precision of conclusions.<sup>2</sup> The analysis of decomposition to estimate the Postmortem Interval (PMI) is one of the important functions of forensic anthropologists; yet, analytical methods that rely on the judgment of human observers, such as assessing PMI from decomposition, may be vulnerable to cognitive bias, leading to inaccurate results. To date, there is a lack of research examining the effects of cognitive bias on decomposition scoring methods and on what specific cognitive factors are most predictive of the decision-making process. Therefore, the goal of this project is to understand how bias might impact TBS values and the role played by cognitive factors, such as the examiners' mood and motivations, on the analysis of decomposition characteristics in the field.

Two experimental trials were conducted whereby the TBSs of four experts with extensive knowledge and experience with the human decomposition process were compared with those of 50 inexperienced undergraduate observers. Each group scored ten donated individuals daily between October 2016 and April 2017 at the University of Tennessee, Knoxville's Anthropology Research Facility. Observers also completed two psychological measures designed to assess their motivations (Intrinsic Motivation Inventory) and mood (Positive and Negative Affect Schedule) after each scoring session.<sup>3,4</sup> Hierarchical random intercept multiple regression models were conducted to assess the relationship between experts' and observers' TBS values as well as the cognitive measures and observers' TBS scores. These models revealed significant differences in TBS values between observers and experts in both trials ( $p < 0.05$  in both trials). However, the directionality of the difference depended on the unique contextual information present in each trial. These hierarchical models were also utilized to assess which cognitive factors, if any, might be predictive of observers' TBS values. Overall, observers' scores were predicted by different factors in each trial. In Trial 1, how nervous observers felt while scoring ( $p < 0.0001$ ), how much they enjoyed scoring ( $p < 0.05$ ), and negative mood ( $p < 0.05$ ) were all predictive of TBS. However, in Trial 2, only observers' perceived scoring ability (i.e., competence;  $p < 0.001$ ) was a significant predictor, due to observers' previous experiences in Trial 1.

Overall, results indicated that bias does exist within the TBS method, with scores depending on the specific contextual information available at the scene as well as the experience and motivations of the observer. This study builds upon previous research examining bias within biological profile methods in forensic anthropology, and it has the potential to impact standard protocols when utilizing the TBS method during longitudinal decomposition research conducted at outdoor decomposition facilities.

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#### Bias, Human Decomposition, Total Body Score



### A85 Evaluation and Minimization of Contextual Bias in Forensic Anthropological Casework in the Context of a Medical Examiner's Office

*Julie M. Fleischman, PhD\**, Harris County Institute of Forensic Sciences, Houston, TX 77054; *Michal L. Pierce, MS*, Harris County Institute of Forensic Sciences, Houston, TX 77054; *Christian Crowder, PhD*, Southwestern Institute of Forensic Sciences, Dallas, TX 75207

**Learning Overview:** After attending this presentation, attendees will better understand the sources of bias that affect forensic anthropological casework in the context of a medical examiner's office and how to reduce the introduction of biasing data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing recommendations for mitigating potential issues of bias in forensic anthropology. This research illustrates the significance of evaluating sources of contextual bias and acknowledging the changes that can be implemented to promote less biased analyses.

In recent years, forensic scientists have given more attention to the presence of bias during scientific examination. Cognitive bias refers to modifications of judgment resulting from the presence of internal expectations and external information. Because it influences decision-making and interpretations, cognitive bias—particularly confirmation bias—must be critically evaluated and minimized, when feasible. Several studies have been published demonstrating the evaluated effects of contextual information on forensic anthropological interpretations of the skeletal biological profile and have found that extraneous information can result in biased conclusions. However, none of the available studies originated from a medical examiner's context. Although these studies identified sources of cognitive bias, they may not capture the nuances of anthropological activities in the medical examiner setting. This research fills this knowledge gap.

The purpose of this project was to identify where and how cognitive bias impacts forensic anthropological analyses conducted at the Harris County Institute of Forensic Sciences (HCIFS). To achieve this goal, the flow of information from the death scene to the laboratory, and within the laboratory, was examined over the course of one year. Subsequent to extended observations and shadowing of HCIFS forensic anthropologists, pathologists, and investigators, the following sources of bias were identified: (1) attending death scenes to locate or recover human remains—law enforcement and investigators often propose theories about the decedent, and contextual information and items, such as clothing and ligatures, are clearly visible; (2) attending case presentation meetings—scene and presumptive identification information are presented at the morning meeting in which daily cases are discussed prior to autopsy; (3) discussions during anthropological consults—discussion with the pathologist during autopsy often reveals contextual information about the case. Sharp force trauma cases, in particular, are of interest because the pathologist frequently notes the precise type of blade that was presumed to have been used (i.e., serrated or non-serrated); (4) radiographic comparisons—anthropologists typically view antemortem and postmortem images simultaneously for the purpose of radiographic identification; (5) language used while discussing unidentified decedents—general use of personal pronouns for an unknown decedent, prior to the anthropologist assessing the remains to estimate sex, has been observed; (6) peer review—the peer review process, particularly for radiographic identifications, does not include blind verification; and (7) information management systems—knowledge of a decedent's tentative identification and case information is accessible by staff via the agency's electronic information management system.

Cognitive bias and the presence of extraneous contextual information cannot be eliminated in a medical examiner's office, particularly one in which various forensic scientists work side-by-side. However, one goal of this project was to provide recommendations for mitigating the introduction of bias into anthropological casework. The following recommendations draw on various suggestions and methods provided by prior researchers, but with an emphasis on medical examiner-specific context: (1) the anthropologist who attended the scene does not conduct the skeletal analysis; (2) the anthropologist responsible for the day's cases does not attend the morning meeting; (3) evaluate antemortem and postmortem radiographs for identification independently, prior to comparison; (4) avoid using personal pronouns for unidentified decedents; (5) the peer reviewer should independently assess specimens and data and form general conclusions prior to reading the analyst's report; (6) anthropologists should avoid accessing contextual case information prior to the examination of the remains; and (7) record in bench notes what contextual information was relayed prior to beginning examination.

Although cognitive bias is inherent in human decision-making, evaluating and mitigating it in forensic anthropological casework is necessary to provide objective scientific conclusions. This research assessed sources of cognitive bias in the context of a medical examiner's office and provides recommendations for forensic anthropologists to reduce bias. While not all forensic anthropologists work in a medical examiner setting, these recommendations are useful tools for all practitioners.

#### **Bias, Medical Examiner's Office, Contextual Information**



### A86 Forensic Anthropology Down Under: Reflections on the Development and Practice of Forensic Anthropology in Australia

*Soren Blau, PhD, Melbourne, Victoria 3146, AUSTRALIA; Samantha K. Rowbotham, MArSc\*, Victorian Institute of Forensic Medicine, Southbank, Victoria 3006, AUSTRALIA*

**Learning Overview:** After attending this presentation, attendees will understand the context in which forensic anthropology is practiced in Australia.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an improved understanding of the political and geographical context in which forensic anthropology is practiced and researched in Australia, and how such practice and research fits into the global development and advancement of the discipline.

This presentation provides an overview of the development and current practice of forensic anthropology in Australia. Similar to the development of forensic anthropology in many countries, the growth of forensic anthropology in Australia over the past century is closely linked to the history of anatomy and physical anthropology. Although relatively slow to develop, forensic anthropology is now a well-established discipline and is seen as complimentary, rather than supplementary, to forensic medical investigations.

There are currently six professionally endorsed forensic anthropologists covering the six states of Australia. These anthropologists form the Forensic Anthropology Scientific Working Group (FA SWG), which is the professional body of forensic anthropology practitioners in Australia as endorsed by National Institute of Forensic Science. Under the FA SWG, national guidelines for forensic anthropology practice have been established. The various contexts in which Australian forensic anthropologists contribute to medicolegal cases are diverse and involve collaboration with other forensic medical specialists and law enforcement personnel. Such contexts include: locating and recovering human remains; examining domestic cases of skeletonized remains; consulting with pathologists on complex cases of skeletal trauma; contributing to the development of a national missing persons database; working with Indigenous communities for repatriation and reconciliation; working with the Australian Army to recover and identify Australian war dead; recovering and identifying human remains in national and international Disaster Victim Identification situations, and undertaking deployments for international humanitarian organizations, especially for the neighboring Asia-Pacific region, but also the larger global context.

Although Australia has a professionally recognized body of forensic anthropologists, there is no formal accreditation process currently available to Australian anthropologists that is like accreditation processes in North and South America and Europe. The issue of accreditation is currently being reviewed to ensure Australian forensic anthropologists are on par with their international colleagues.

Research in forensic anthropology has significantly expanded over the past 20 years with the development of new resources that allow anthropologists to address relevant questions both in the Australian and international contexts, as well as in associated forensic medical disciplines (e.g., odontology and entomology). These resources comprise clinical radiograph and computed tomography collections within the country's major hospitals; the postmortem computed tomography collection (Victorian Institute of Forensic Medicine); the Australian Facility for Taphonomic and Experimental Research (University of Technology Sydney), and the School of Biomedical Sciences Skeletal Collection (University of Queensland). These resources have enabled substantial advancement in the development of population-specific standards for the modern Australian population, have strengthened current understandings of trauma analysis and interpretation, and have also facilitated additional work on estimations of the postmortem interval.

The development of these resources for research has also contributed to a substantial increase in undergraduate and post-graduate student enrollments across all Australian states and territories, with universities increasingly offering forensic anthropology as a specialization within formal anthropology and biological science studies. This increase in students has also recently seen the development of the first master's degree specifically in forensic anthropology (University Of Western Australia) in Australia.

The influence of geography, and therefore the political context, in which forensic anthropologists work in Australia reflects the nature of the anthropologists' involvement in medicolegal cases. This involvement, and subsequent development of the discipline, shows both similarities and differences to how forensic anthropology is practiced across the globe.

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#### Forensic Anthropology, Australia, Context



### A87 Forensic Anthropology: A View of Brazil

Yara V. Lemos, MS\*, Laboratory of Forensic Anthropology, Belo Horizonte, Minas Gerais 30510160, BRAZIL

**Learning Overview:** After attending this presentation, attendees will be familiar with the work, resources, training, educational requirements, research advances, and legal grounds of forensic anthropology in Brazil and, more deeply, in the state of Minas Gerais.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the work of forensic anthropologists outside the United States, especially by emphasizing the performance of Brazilian professionals and how they balance demands and resources. This presentation will also help create a channel for professionals to discuss and debate the successes, challenges, and possible solutions for maintaining the high standards of the field worldwide.

Brazil is one of the most violent countries in the world. The challenges Brazilian forensic scientists and, specially, forensic anthropologists struggle with are substantial. It is reasonable that the forensic community shall look at those who dare to face this reality daily, with diligence, dedication, and devotion.

The Brazilian Penal Process Code limits forensic investigation of crimes to official experts. Of those, primarily official medicolegal experts and dental experts work in the forensic anthropology services. Although there are other fields of knowledge and graduations that could apply to the service, such as biology, physical anthropology, and archaeology, those are more active as academic researchers, museums curators, and university professors.

Regardless of their degree, there is mandatory training for forensic science in Brazil, which varies from state to state. Further dissimilarities, comprising financial and material resources, income, and workplace, can be seen between the states. Situated in the capital, as a branch of the Legal Medical Institute, the Forensic Anthropology Laboratory of Minas Gerais (LAF) was founded in the 1980s. It comprises a multidisciplinary team of eleven experts, composed of eight medicolegal experts and three dentistry experts. The LAF's greatest challenge is to deal with casuistic and complex murder cases, such as microwave-processed bodies and aircraft accidents. The work is always interdisciplinary, and complementary resources such as anatomopathological exams, digital radiographic exams, computerized tomography, DNA tests, and toxicological screen are available.

The peculiarities of medicolegal examinations of human remains in advanced states of putrefaction, notably skeletonized corpses and charred bodies, shifted a growing scientific evolution, and LAF sought help from literature and scientific journals to keep the procedures up to date. Scientific productions and developments in Brazil take place primarily at universities, where the research funds and incentives are concentrated. There are post-graduation programs in forensic science, in addition to forensic anthropology and archeology centers, which have taken place in this century.

There are seven identified osteological collections in the country, mainly housed in universities, pointing to advances in the field of forensic anthropology and other associated forensic science disciplines.<sup>1</sup> These modern, documented skeletal reference collections provide access to osteological laboratory research for forensic scientists to conduct innovative interdisciplinary research. Recently, the creation of the Brazilian Association of Forensic Anthropology (ABRAF) propitiated interactions between the existing experts in the nation and abroad in annual scientific meetings.<sup>2</sup>

Brazil has vast research and outreach potential for the medicolegal and forensic science communities. It is important that researchers are aware of the available resources that can assist the evolution of the biological profile, taphonomy, and human identification in the forensic context.

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2. ABRAF, Brazilian Association of Forensic Anthropology. Accessed July 25, 2018. <http://antropologiaforense.org.br>.

#### Forensic Anthropology, Opportunities, Brazil



## A88 Forensic Anthropology and the Philippines

Matthew C. Go, MA\*, Department of Defense POW/MIA Accounting Agency, C, Joint Base Pearl Harbor-Hickam, HI 96853

**Learning Overview:** The goal of this presentation is to qualitatively appraise the state of forensic anthropology in the Philippines in terms of its academic, legal, and practical capacity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the unique challenges and possible solutions faced when working in developing countries with limited local forensic capacity yet high endemic caseloads.

Forensic anthropology, despite its relatively recent maturation as a discipline and obscurity among the lay public, has grown to serve a global need within the medicolegal sphere. However, the availabilities of qualified experts, opportunities for education, research, and training, and material necessities such as facilities, equipment, and funding are not equitably distributed across the world. Moreover, countries that often encounter the heaviest forensic anthropological caseloads also have the least amount of operational resources. This presentation is part of an invited symposium entitled “International Forensic Anthropology,” which features the different manners in which forensic anthropology is developed and practiced around the world. Here, the development and application of forensic anthropology in the Philippines in both the academic and applied domains is reviewed.

The Philippine context, as well as the Asia-Pacific region more generally, provides a prime case for the need for forensic anthropology. In the past decade, the Asia-Pacific region experienced 40% of the world’s natural disasters and 80% of the world’s disaster-affected populations.<sup>1</sup> The Philippines, ranking third for most disaster-prone country, regularly falls victim to typhoons, earthquakes, landslides, and floods, in addition to man-made disasters, such as transportation, industrial, and fire accidents.<sup>2</sup> The country has also experienced and continues to struggle with episodes of armed conflict, terrorism, and human rights abuses. These factors, coupled with a large endemic population size, widespread diaspora, high poverty rate, and tropical environment, result in a substantial volume of forensic caseloads that could benefit from anthropological input.<sup>3</sup>

Within the academe, there are currently no programs that are well equipped to train biological, let alone forensic, anthropologists. This has been in large part due to a lack of specialized faculty and, until recently, adequate reference material. The past three decades have seen less than ten papers on Philippine forensic anthropology published, with most of these only resulting from the recent amassment of a modern Filipino skeletal reference collection.<sup>4</sup> Undergraduate programs in forensic science are rapidly gaining popularity, with forensic anthropology remaining as an undermanned elective.

Within legislative bodies, several proposed bills have implications for both the academic and practical progress of forensic anthropology. Many of these bills move to improve death investigation guidelines in the Philippines and establish a national institute for the forensic sciences. However, these proposals have seen little progress through the legislative process, while implementation, once passed, sees its own set of challenges. Moreover, no regulatory body provides quality assurances in the investigation process nor in the qualifications of individual analysts.

Within applied settings, licensed physicians sworn in as police officers manage the ambit of medicolegal investigations. For daily casework, there are no anthropologists employed full-time or as consultants in any of the responsible governmental bodies, namely the Philippine National Police, National Bureau of Investigation, Commission on Human Rights, or the public attorney’s office. International humanitarian assistance and anthropological training have been offered for high-profile mass casualty contexts such as Typhoon Haiyan, the Marawi Conflict, and cases of the disappeared from the Marcos regime, but sustainable approaches to integrating anthropology have been lacking. Cases of state-sanctioned violence also present major conflicts of interest when almost all death investigations are overseen by police officers.

While the Philippines stands to gain much from developed academic programs that produce both able anthropologists and well-researched population standards and best practices, the country must also provide the appropriate medicolegal framework in which anthropologists may operate. This work takes stock of the scale, infrastructure, and political will of the Philippine context in that it may provide Philippine forensic anthropology its due appraisal.<sup>5</sup>

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### Forensic Anthropology, Local Capacity, Philippines



### A89 An Anthropology Roadmap: Sri Lanka

*Sunil P.A. Hewage\*, Colombo 10, Western Province, SRI LANKA*

**Learning Overview:** After attending this presentation, attendees will better understand the current status of forensic anthropology in Sri Lanka.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the historical background, current status, and future developments of the field of forensic anthropology in Sri Lanka, opening a channel for discussion and comparison between other nations.

The history of forensic medicine in Sri Lanka goes back to the inception of Western medical practice by the British rulers in the 18<sup>th</sup> century. Forensic medicine practitioners, referred to as Judicial Medical Officers (JMO) in this era, took the responsibility of handling forensic anthropology casework since there were no trained forensic anthropologists in Sri Lanka. In those days, Sri Lanka was a comparatively peaceful country, the forensic anthropology case load was very minimal, and most of these cases were limited to partially or completely skeletonized bodies found in abandoned areas probably due to suicides or animal attacks. Ordinary medical officers attached to government hospitals handled most of these uncomplicated cases with their limited knowledge gained during undergraduate training and experience gained by handling these types of cases. The few forensic experts (consultant JMOs) who were trained in the United Kingdom took the responsibility of handling complicated issues of forensic anthropology in this era.

The Post Graduate Institute of Medicine (PGIM) established in 1980 took over the responsibility of training local forensic experts in Sri Lanka. Analysis of skeletal remains was incorporated into the curriculum of both post graduated Diploma in Legal Medicine (DLM) and Doctor of Medicine (MD) training programs since 1980. Those who completed the MD Forensic Medicine program possessed a reasonably good knowledge of forensic anthropology, and this was further improved during their post-MD overseas training. Attending workshops and seminars conducted by overseas forensic anthropologists and international seminars and workshops in forensic anthropology helped them to further improve their knowledge in this field.

On August 23, 2016, the Sri Lankan Parliament passed the Act to establish an Office of Missing Person (OMP). The purpose of this act was to investigate people who disappeared during the 30-year-long civil war that prevailed in Sri Lanka until 2009. Furthermore, the Sri Lankan government recognized the need for an expert to oversee the forensic anthropology works in the country. Therefore, the Ministry of Health in Sri Lanka appointed a Consultant Judicial Medical Officer (JMO) to the Institute of Forensic Medicine & Toxicology (IFMT) Colombo, the premier medicolegal institute of Sri Lanka. Responsibilities of the Consultant JMO appointed to this new post are as follows: (1) take the responsibility of investigating skeletal remains/partially skeletonized bodies recovered from all over the country in order to establish the identity, cause, and manner of death, and other relevant issues, and report to the court of law; (2) coordinate the forensic work with the OMP and be involved in investigations of mass graves, if found; (3) improve the existing anthropology lab at IFMT in order to carry out the skeletal analysis; and (4) set up a museum in order to train both undergraduate and postgraduate trainees.

Additionally, the PGIM has taken an interest in training more post-MD trainees in forensics, which will be conducted during the post-MD overseas training. Service of the first trainee trained in forensic anthropology will be available by the end of 2019. Specialists trained in this manner will be appointed to various parts of the country. Thus, in the future, Sri Lanka will have a sufficient number of trained forensic anthropologists with a forensic medicine background.

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#### Forensic Anthropology, Sri Lanka, Current Status





### A90 Forensic Anthropology As a Neglected Field of Inquiry in Forensic Death Investigations in India: A Critical Evaluation of the Current Scenario and Future Possibilities

*Jagmahender Singh Sehwat, PhD\*, Panjab University, Dept of Anthropology, Chandigarh 160014, INDIA*

**Learning Overview:** The goal of this presentation is: (1) to inform attendees about the current status and future prospects of utilization of anthropological knowledge in forensic death investigations in India; and (2) to seek suggestions from international participants on how to enforce forensic anthropological examination of unknown skeletal remains as an integral part of such analyses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that forensic anthropological analyses should be considered as a significant component in forensic death investigations of unknown human remains.

Forensic anthropological evidence can be used more effectively and robustly to help the criminal legal system in a country such as India where subtle variations in human physical and skeletal features of diverse heterogeneous population groups can be useful for identification of unknown individuals/skeletal remains. Investigating agencies generally seek the assistance of forensic pathologists for identity establishment of bundles/heaps of unknown human skeletal remains retrieved from desolate forests, natural ravines, roadside trenches, disused wells, potholes, sewage systems, pits, waterways, etc. The inherent medical expertise of a pathologist trained in India is not considered sufficient to opine an objective identity of such remains. In India, a forensic anthropologist is rarely warranted for the identification of such remains, as there are hardly any Indian medical institutions where forensic anthropologists are employed as experts in the constituted multidisciplinary investigating teams.

Forensic anthropologists are supposed to have in-depth specialized knowledge of human bodily features and skeletal variations present among individuals of different population groups. There are several criminal cases reported in India in which forensic anthropologists have played a crucial role in identification strategies of the victims or suspects, such as: the Sheena Bora murder (Mumbai, 2012), the Nirbhaya gang-rape (Delhi, 2014), the 26/11 Mumbai terror convict (Mumbai, 2008), the Nithari serial rapes and killings (Ghaziabad, 2006), and the Naina Sahni Tandoor murder (Delhi, 1995) case. Very recently, thousands of human skeletal and dental remains, in addition to many contextual items of personal identity, have been recovered from an abandoned well found situated beneath a religious structure in a north Indian state.

The deep sense of professional superiority has seriously affected better dispensation of justice in India as anthropological analysis has not been considered an essential component of medicolegal death investigation by forensic pathologists. The medicolegal fraternity doubt and ignore the significance of vital anthropological input in forensic death investigations, particularly in mass disaster cases where multiple casualties occur. India is home to mass disasters (both natural and man-made) wherein thousands of people die nearly every year. The identity establishment of such victims becomes essential for the completion of some documentation, such as insurance claims, job benefits, property claims, bank transfers, etc.; however, they remain unsolved puzzles for years, despite the best efforts of forensic pathologists (without any expertise inputs from anthropologists). The unknown human skeletal remains brought by police officials are viewed as physical objects and such autopsies are conducted by non-forensic clinicians for the sake of completion of some legal formalities. Thus, vital clues of identity about the victims are lost at the postmortem table in the absence of an expert opinion of a forensic anthropologist in India.

Forensic anthropologists can look over the shoulders of a forensic pathologist at the autopsy table for the most reliable and scientific identification acceptable in legal contexts, so the experts of two disciplines should adopt a collaborative approach. This presentation will highlight the current scenario of the utilization of anthropological knowledge in forensic death investigations in an Indian context and its future possibilities in reference to recent forensic cases solved utilizing anthropological input and evidence. Conclusions have been derived because of experiences working as an anthropologist for several years in a forensic medical department of a medical institution and by interviews of forensic pathologists of various autopsy departments across the country.

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#### Forensic Anthropology, Current Status, India



### A91 Forensic Anthropology Practice in Indonesia

*Evi Untoro, MD\*, Sentra Medika Cibinong, Hospital & Funeral Home, Bogor, West Java 16911, INDONESIA*

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**Learning Overview:** The goal of this presentation is to highlight the diverse background and challenges that professionals working with forensic anthropology face in different countries. The long history of forensic anthropology has brought considerable advances, and it is essential that these advances are noticed, and trends assessed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by creating a channel for professionals to discuss and debate the successes, challenges, and possible solutions to maintain the high standard of the field worldwide.

Indonesia belongs to the South East Asian Region, where its position at the archipelago provides a hot tropical climate and high humidity. Most often, the forensic cases that are found at the scene of open ground are skeletal remains. Due to the area's religion (approximately 80% of the population is Muslim (Islamic Religion)), their belief from the Quran is that the dead body should be buried immediately (before 24 hours) and the sooner the better. However, with a police investigation for unnatural death, this is becoming an obstacle and often after burial, the family will request an investigation and autopsy, with some remains being skeletal. This task is performed by forensic pathologists in a big city where there are forensic departments in institutions or hospitals, but in places where there is no forensic pathologist, the police will sometimes ask the physical anthropologist to help them solving this problem.

Indonesia does not have a forensic anthropology department, only a division from the faculty. After graduating from the faculty of medicine as a General Practitioner, a person can choose to become a forensic pathologist with three to four years of training, including two semesters of forensic anthropology, where they learn anatomy, biology, and the pathology of skeletal remains. Physical anthropologists can graduate from any discipline and then choose to study sociocultural anthropology, including some forensic anthropology/physical anthropology into their faculty curriculum for one semester.

The forensic pathologists that provide the forensic anthropology examination should also having a good quality education and advanced training to make the best report. The physical anthropologist should be guided by a forensic pathologist who is practicing forensic anthropology to share and transfer knowledge between them, especially in looking for forensic evidence to solve cases.

Within these global cases, the highest standards of examination and reporting procedures should be used. This panel presentation would like to combine the advance methods and highest quality of training when practicing forensic anthropology fieldwork collaborations with the international forensic anthropology association and institutions, as well as universities.

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### Forensic Anthropology, Training and Practice, Indonesia



### A92 Forensic Anthropology Research Centre (FARC): An Emerging Community of Practice in South Africa

*Ericka N. L'Abbe, PhD\**, University of Pretoria, Pretoria 0001, SOUTH AFRICA; *Leandi Liebenberg, MS*, Pretoria 0186, SOUTH AFRICA; *Gabriele C. Kruger, MSc*, Pretoria, Gauteng 0084, SOUTH AFRICA; *Anna C. Oettle, MBChB, PhD*, Sefako Magatho University, Ga-Rankuwa 0204, SOUTH AFRICA; *Steven A. Symes, PhD, MS Medical Examiner's Office, MCL, Pearl, MS 39208*; *Shari A. Eppel, BA, Erie, PA 16504*; *Kyra E. Stull, PhD, University of Nevada, Reno, Reno, NV 89557*; *Marie Christine Dussault, PhD, Brampton, ON L6Z 4B6, CANADA*; *Michael W. Kenyhercz, PhD, Department of Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI 96816*; *Yvette Scholtz, MSc, University of Pretoria, Arcadia 0084, SOUTH AFRICA*; *Clarisa Sutherland, BSc, Pretoria, Gauteng 0083, SOUTH AFRICA*; *Alieske C. Hagg, MSc, Pretoria, Gauteng 0028, SOUTH AFRICA*

**Learning Overview:** After attending this presentation, attendees will better understand the emerging practice in South Africa (SA) of the analysis of unidentified skeletal remains, establishment of skeletal collections, education, research, and community service.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the knowledge of education and training in forensic anthropology in Africa, particularly in South Africa.

The FARC was established in 2008 with the goal of producing research and education on par with international standards on all aspects concerning the identification of human skeletal remains. Each year in Gauteng, approximately 1,200 bodies are incinerated without a known identity, many of whom may be illegal immigrants and/or migrant laborers from other provinces in the country or the African continent.<sup>1</sup> Gauteng is one of nine provinces in SA, and the total number of missing and unidentified persons within the entire country is unknown. The forensic anthropology laboratory of FARC receives anywhere from 80 to 100 unknown persons from the Forensic Pathology Services (FPS) and the South African Police Services (SAPS) each year. If a person is not identified after analyses and with further investigations from the SAPS and the Victim's Identification Centre (VIC), the skeletal remains are accessioned into archival storage until they are identified. Historical and geographical circumstances (migrant labor) and illegal entry into the country confounds identification in the country. The purpose of this presentation is to discuss the contribution of FARC and its collaborators in developing and improving education, research, and community engagement in forensic anthropology in SA within the past ten years.

FARC has contributed to improving proficiency and expertise among practicing SA forensic anthropologists with the development of freely available, population-specific databases; the introduction of standard operating procedures in forensic anthropology laboratories at the various universities; and the recent provision of annual proficiency testing for practitioners. Expertise development starts as early as the undergraduate years and continues into the practitioner's workplace. For this reason, undergraduate and postgraduate physical/forensic anthropology curricula have been modified with the goal of encouraging critical and relativistic thinking among students and of providing hands-on experience with cold cases and course facilitation. Structured, mentored experiences are designed at all levels of education to prepare students for the real world of work, which is not limited to academia.

FARC-affiliated researchers from South Africa, Europe, and the United States publish between 10 and 20 manuscripts and book chapters a year on a range of physical anthropology topics, including human and hominin variation, cranial-facial comparisons, periodontics, and bone trauma. National and international researchers obtain data from the Pretoria Bone Collection, a modern 20<sup>th</sup>-century South African skeletal collection that houses approximately 1,700 remains. In collaboration with various South African and European partners, current research plans are underway to establish a diverse, large, and digitally available imaging repository of various skeletal collections and anatomical specimens. The goal is for this to place South African forensic anthropology at the cutting edge of collaborative scientific research; improved internationalization of SA with an increase in postgraduate students, as well as competitive undergraduate learning facilitation; and to improve qualifications of academic staff in the country.

Community engagement has grown in the past ten years, adding value and applicability to the consultation services with the SAPS, forensic pathologists, and other humanitarian forensic-based training/research projects in Africa. For the past two years, FARC members have participated in training six Zimbabwean anthropologists from the Ukuthula Trust, a Non-Governmental Organization (NGO) founded in Zimbabwe in response to the Gukurahundi massacres in Zimbabwe and are also forming research and training collaborations with East African colleagues (Tanzania).

The contributions from forensic anthropologists in research, education, and community engagement are both sustainable and relevant within the South African community. FARC is committed to promoting excellence in research, teaching, and training both at UP and other tertiary institutions; this particularly includes improving proficiency of forensic anthropologists in the country, as currently no guidelines exist in defining this professional discipline, and to assist in training the next generation of forensic anthropologists to work on the African continent within a strong community of practice.

#### Reference(s):

<sup>1</sup>. Wild S. 2016. <http://mg.co.za/article/2017-01-12-00-long-quest-to-understand-these-bodies-without-identities>.

#### Proficiency, Student Training, Research



### A93 Forensic Anthropology in Greece: An Overview of the Discipline's Status

*Elena Kranioti, PhD\*, Edinburgh Unit for Forensic Anthropology, Crete, GREECE; Konstantinos Moraitis, PhD\*, University of Athens, School of Medicine, Athens 11527, GREECE*

**Learning Overview:** After attending this presentation, attendees will have gained an insight into forensic anthropology practices in Greece.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comprehensive description of the education, research, and practice of the forensic anthropology discipline in Greece, a country faced by increasing challenges in forensic identification in mass fatalities, such as the recent shipwrecks in the Aegean and wildfires on the mainland.

Forensic anthropology is a fast-growing discipline with numerous applications in medicolegal contexts. Among many European countries exists certain variability in the educational system, research focus, and actual casework. This presentation seeks to present an overview on the status of forensic anthropology in Greece with the goal of informing young forensic practitioners and the broader medicolegal community on the advances and limitations of the discipline in a challenging geographic area with often-increased needs for specialized forensic services.

The foundation of forensic anthropology in Greece began with the establishment of the Forensic Anthropology Unit (UoAFAU) at the Department of Forensic Medicine and Toxicology of the National and Kapodistrian University of Athens in 2008. The Unit has been operating ever since under the directorship of the only employed forensic anthropologist in Greece who holds an Associate Professor's position. The Unit, which is fully equipped with maceration facilities and analytical tools, provides anthropological examinations at the request of the Court, Prosecution Office, or police. In addition to the usual forensic cases involving crime scene investigation, the establishment of a biological profile, personal identification, evaluation of skeletal trauma, and estimation of the postmortem interval, the Unit also undertakes examinations aimed at estimating the age of living individuals. Furthermore, the Unit provides training to graduate and postgraduate students of different universities, as well as training to forensic pathology residents at the University of Athens. Forensic anthropology services are also provided by trained forensic pathologists with postgraduate degrees in forensic anthropology at the University of Crete and Ioannina. These professionals are employed as forensic pathologists, though, and they deal with forensic anthropology as part of their routine casework; thus, their anthropological report is incorporated into the routine autopsy report and does not comprise a separate document. The protocols followed in each institution are mainly subject to available equipment and resources. For example, the University of Crete is not equipped with a maceration chamber, but instead heavily decomposed remains can be examined through Postmortem Computed Tomography (PMCT) as part of the Virtopsy.GR research project. Thus, the examination protocol of heavily decomposed remains includes a routine PMCT examination, which is evaluated by a forensic pathologist trained in forensic anthropology and virtopsy techniques. The main guidelines followed by forensic anthropology professionals in Greece include the recommendations of the American (Working Group for Forensic Anthropology) and European (Forensic Anthropology Society Europe) standards, as currently there is no accreditation system for forensic anthropology practitioners.

The limited number of forensic anthropologists in the country hinders the development of a robust educational system of forensic anthropology in Greece. Most graduates are seeking further training outside the country and primarily end up performing postgraduate training in the United Kingdom. Most universities in Greece offer a PhD option in a Forensic Anthropology topic resulting in an increase of research outputs in the last decade. The advances in research are further enhanced with the creation of two modern, documented skeletal collections in Athens (2003) and Crete (2005), which served as research material for numerous population-specific and other types of studies.

The increased number of mass incidents in Greece (immigration crisis, fires, etc.) calls for employing more forensic anthropology experts in the medicolegal system. In addition, the reorganization of the Greek Disaster Victim Identification (DVI) operational procedures since 2017 highlighted the need for trained forensic anthropologists to equip the new DVI teams. It is hoped that the presentation of the current status of forensic anthropology in Greece will serve as a cornerstone in the promotion of the discipline at educational and professional levels, so it can meet the increased needs of the country.

#### Forensic Practice, Greece, Forensic Anthropology



### A94 Routine Practice in Forensic Anthropology in Europe: Attempting to Achieve a State of the Art

*Eugenia Cunha, PhD\**, National Institute of Legal Medicine and Forensic, Coimbra, AB 3000-456, PORTUGAL; *Elena Kranioti, PhD*, Edinburgh Unit for Forensic Anthropology, Crete, GREECE; *Pascal Adalian, Marseille, FRANCE*; *Eric Baccino, MD, Hopital Lapeyronie, Montpellier, Cedex 5 34295, FRANCE*; *Cristina Cattaneo, PhD, LABANOF - Sezione di Medicina Legale, Milan 20133, ITALY*

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**Learning Overview:** The goal of this presentation is to present the reality of practicing forensic anthropology in Europe, 15 years after the creation of the first scientific association dedicated to this discipline, the Forensic Anthropology Society of Europe (FASE).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the reality of practicing forensic anthropology in countries such as Portugal, Spain, Italy, France, Greece, Hungary, Denmark, Belgium, Hungary, and The Netherlands. This presentation will assess whether these practitioners are ready to face the new challenges of the current global society.

**Hypothesis:** It is hypothesized that despite the different backgrounds of the practitioners, from medicine to anthropology to biology, it is expected that the same guidelines are being followed by the experts on this field. This is due to the efforts of the FASE, namely through the realization of annual workshops and/or courses and certification processes.

**Statement of Methods:** Each European country will be detailed in terms of educational background, number of employed forensic anthropologists or number of forensic practitioners who employ forensic anthropology methods, and approximate number and context of cases. Throughout the presentation of some practical cases from different European countries, highlighting the methods, the complementary exams regularly made, and the reports, this presentation seeks to demonstrate the reality of this discipline and how it interacts with other medico and forensic areas.

**Statement of Results:** Many forensic anthropologists in Europe have a medical background and often specialize in forensic medicine. Research is undertaken by many diverse scientists with backgrounds ranging from archaeology and osteoarchaeology to anatomy, biology, physical anthropology, dentistry, and medicine. Research outputs have noted an impressive increase over the past two decades, and most European countries have acquired population-specific data for forensic application at some level. The three main contexts in which forensic anthropology is practiced in most of the European countries is the forensic investigation of heavily decomposed human remains, the positive identification of unidentified human remains, and the age estimation of living individuals. Accreditation is possible at a European level through the FASE examination process.

**Conclusion:** It is evident that the different legislation in European countries has an impact on the routine practice of forensic anthropology and its role in the courtroom. FASE's continuous efforts in continuous professional education and dissemination of good practice is hopefully a positive contribution toward achieving a normalization of forensic anthropology practice throughout Europe.

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### Forensic Anthropology, Practice in Europe, Expert Report



### A95 The Use of a Gelatin-Based Consolidant to Preserve Thermally Altered Remains

Jan J. Topoleski, MFS\*, Quantico, VA 22135; Angi M. Christensen, PhD, FBI Laboratory, Quantico, VA 22135

**Learning Overview:** After attending this presentation, attendees will be familiar with a novel method of using a gelatin-based consolidant for preserving and recovering thermally altered remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a possible new method to protect and preserve the relative anatomical positioning of thermally altered remains during recovery and transport.

Human remains from fire scenes can be severely altered, including being highly fragmented and extremely fragile. Recovery, preservation, and transport can therefore be very challenging in these cases due to the potential for the remains to become further fragmented during the recovery process. Few methods of preserving burned skeletal remains have been scientifically tested or generally accepted by crime scene investigators and forensic analysts. This study tests whether the application of commercially available food-grade gelatin as a consolidant may be an effective method for preserving thermally altered skeletal remains. Food-grade gelatin was selected due to being easily available and non-reactive (i.e., will not chemically or physically alter bone).

Twelve domestic pig (*Sus scrofa*) mandibles were each placed into aluminum trays filled with sand. The specimens were thermally altered using a hand-held propane torch (temperatures >1,100°F), which was applied to each bone for ten minutes. Distance and position of the heat source were kept constant to ensure similar thermal exposure to all specimens. The mandibles were allowed to cool *in situ*. Six of the mandibles were then treated with a 1:10 mixture of Knox® brand food-grade gelatin and warm water using a hand-held spray bottle; the other six mandibles remained untreated. The gelatin was then allowed to cure for 60 minutes.

Specimens were each removed/recovered from the sand substrate manually and transported approximately 14.5' where they were then assessed and photographed. Preservation was assessed as a function of the total number of fragments following transport, with better preservation being represented as fewer fragments.

Results demonstrate that treated samples were significantly better preserved (i.e., were represented by fewer fragments following transport) compared to untreated controls. Most of the treated specimens, in fact, remained as one intact piece, along with part of the sand substrate, during the recovery and transport process. In contrast, untreated controls became separated into multiple bone fragments during recovery and transport. Because they remained intact and in anatomical position, the treated specimens could also be visually and radiologically assessed while still held together by the gelatin.

Additional testing of Knox® gelatin (or chemically similar products) for skeletal preservation would be beneficial, including testing the approach in different environments (e.g., varying temperature, humidity) as well as varying degrees of thermal alteration. However, preliminary tests suggest that the application of a gelatin-based consolidant appears to have significant potential as a method for preserving thermally altered remains for recovery and transport. This approach is also easy to use, affordable, non-destructive, and reversible (the gelatin can be removed using warm water if needed). It may also have utility in non-forensic anthropological contexts, including the preservation of archaeological or non-skeletal material and artifacts.

#### Forensic Anthropology, Skeletal Preservation, Gelatin

### A96 The Application of X-Ray Photoelectron Spectroscopy (XPS) to Examine the Surface Chemistry of Cancellous Bone and Medullary Contents to Refine Bone Sample Selection for Nuclear DNA Analysis

Janna M. Andronowski, PhD\*, University of Akron, Akron, OH 44325-3908; Amy Z. Mundorff, PhD, University of Tennessee - Anthropology, Knoxville, TN 37996-1525; Jon Davoren, Lorton, VA 22079-2625; Reed A. Davis, MSc, University of Akron - Department of Biology, Akron, OH 44325; Eric W. Price, PhD, University of Saskatchewan - Dept of Chemistry, Saskatoon, SK S7N 5C9, CANADA

**Learning Overview:** The goals of this presentation are to: (1) describe the use of XPS for surface chemistry analysis of cancellous bone tissue and medullary contents as a novel approach to decipher biological tissues from diagenetic infiltrations (e.g., soil) among trabeculae; and (2) present the methodology as a potential tool for refining bone sample selection for nuclear DNA analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving current understandings of the relationship between nuclear DNA yield and cancellous bone and medullary content chemical composition, thus informing bone-sample selection for nuclear DNA analysis in a forensic context.

Research by Andronowski and colleagues investigated whether differences in 3D bone microstructure may be used to explain differential nuclear DNA yield among bone tissue types (cortical and cancellous bone), with a focus on osteocytes and the 3D quantification of their associated lacunar spaces.<sup>1</sup> Identifying which bone tissue type(s) and/or bone envelope(s) (e.g., periosteal, intracortical, and endosteal) provide the highest nuclear DNA yields will further inform current bone-sampling protocols for human identification and limit the amount of bone tissue necessary for DNA analysis. Results demonstrated that osteocyte lacunar density values were independent of nuclear DNA yield, suggesting an alternative explanation for the higher nuclear DNA yields from bones with high quantities of cancellous bone. A plausible explanation focuses on remnants of soft tissue between trabeculae observed using Synchrotron Radiation-based micro-Computed Tomography (SR micro-CT). Though soft tissue was not present on the surface of the bones, 3D scans consistently revealed probable soft tissues within the medullary cavities of skeletal elements with high cancellous content.

It is hypothesized that residual soft tissues, which likely include endosteum and osteological lining cells, contributed to the higher nuclear DNA yields from cancellous bone. The application of a novel XPS approach has the potential to explain why nuclear DNA yield rates differed among bone tissue types in the specimens used in Andronowski and colleagues original study.<sup>1</sup>

The surface chemical composition of cancellous bone specimens and their medullary contents were assessed ( $n=24$ ) using XPS. Skeletal elements were retrieved from six adult males at increasing postmortem intervals and included the talus, medial cuneiform, patella, and sixth rib. Three Regions Of Interest (ROIs) per specimen were obtained and comparatively analyzed. All XPS experiments were conducted using a Kratos AXIS Supra system at the Saskatchewan Structural Sciences Centre (SSSC). This system is equipped with a 500mm Rowland circle monochromated Al K- $\alpha$  (1486.6 eV) source and combined Hemi-Spherical Analyzer (HSA) and Spherical Mirror Analyzer (SMA). A spot size of hybrid slot (300x700) microns was used. All survey scan spectra were collected in the 5eV–1,200eV binding energy range in 1eV steps with a pass energy of 160eV. An accelerating voltage of 15keV and an emission current of 15mA was used for the analysis. The data was processed using CasaXPS (version 2.3.18PR1.0).

The chemical composition of the surface of cancellous bone and associated medullary contents revealed high percentages of carbon and oxygen (12.33-30.44% and 56.70-85.29%, respectively), which is consistent with biological material. Trace amounts of phosphorous (0.39%-3.98%), calcium (0.34%-6.45%), and nitrogen (1.25%-9.39%) were further detected in all specimens. Sodium, sulfur, and iron were detected in specimens from one individual, albeit in almost undetectable amounts (<0.5%). Most individuals were recovered from the outdoor environment following three and nine months of natural decomposition. However, one individual was placed in a burial and recovered after nearly three years. This individual's bones were visibly soil-stained and revealed trace amounts of silicon (0.71%-1.69%) and aluminum (0.97%). These inorganic elements are the major components of common soil and their presence is likely the result of diagenetic activity. Results indicate that intertrabecular spaces within cancellous bone provide a safe reservoir for soft tissue, suggesting that areas of high cancellous content are a preferential location for nuclear DNA extraction

Results of this work have broader applications as they offer promise for the development of a refined method for identifying the skeletal elements most likely to yield nuclear DNA. The procurement of small, primarily cancellous bones with associated soft tissues within marrow spaces should be preferentially sampled and no longer dismissed as potential DNA sources in favor of cortical bone tissue.

#### Reference(s):

1. Andronowski J.M., Mundorff A.Z., Pratt I.V., Davoren J.M., Cooper D.M.L. 2017. Evaluating Differential Nuclear DNA Yield Rates and Osteocyte Numbers Among Human Bone Tissue Types: A Synchrotron Micro-CT Approach. *Forensic Science International: Genetics*. (28): 211-218.

#### XPS, Nuclear DNA, Cancellous Bone



### A97 Forensic Anthropology and Identification at the State Level: Lessons From the Louisiana Repository for Unidentified and Missing Persons

*Teresa V. Wilson, PhD\*, Louisiana State University, Baton Rouge, LA 70803; Ginesse A. Listi, PhD, LSU Geography & Anthropology, Baton Rouge, LA 70803; Maria T. Allaire, MA, LSU FACES Laboratory, Baton Rouge, LA 70803*

**Learning Overview:** The goal of this presentation is to demonstrate how the partnership between forensic anthropology, investigative agencies, and other forensic professionals in Louisiana has changed the way that unresolved unidentified and missing persons cases are investigated.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by posing a model for a state-level database for cold case unidentified and missing persons investigations.

In 2006, Louisiana passed an unprecedented law (LA RS 15:651-662) that established the Louisiana Repository for Unidentified and Missing Persons Information Program (the "Repository") and outlined the role that forensic anthropology would play in the identification of missing persons across the state. By naming the Louisiana State University Forensic Anthropology and Computer Enhancement Services (LSU FACES) Laboratory the sole administrator of this database, the state legislature recognized that forensic anthropology could provide positive contributions in the collection of biological information from cold cases and was best suited to act as a liaison with other agencies involved in the investigations. After 13 years, the lessons learned from the Louisiana Repository highlight the problems facing efforts to identify the unidentified and find the missing all over the country.

The Repository is a secure, confidential database that contains biological, biographical, and investigative information about Louisiana unidentified and missing persons cases. The earliest versions of the database were a collection of WinID and Microsoft® Excel® and Access® files, which contained specific, but limited, information and was not cross-searchable. In 2015, all Repository files were transferred into a specifically designed, proprietary database program that is housed on a secure off-site server. This upgrade has resulted in more efficient data management by providing a straightforward means of searching for and comparing data. The LSU FACES Lab has maintained a website ([identifyla.lsu.edu](http://identifyla.lsu.edu)) that contains a public interface for global access to basic case information and images since 2008. This website has attracted almost two million page views since it was redesigned in 2010.

For criminal justice agencies in Louisiana, the Repository serves as a central location for archiving information about unidentified and missing persons, as well as provides a means for requesting assistance with other aspects of recent and cold case investigations. As a forensic anthropology laboratory, FACES personnel can provide all Repository cases with anthropological analyses (e.g., biological profile, trauma analysis, postmortem interval, etc.) and forensic imaging (e.g., facial approximation, age progression, etc.). Therefore, in addition to using the Repository to search case information from other agencies within the state, law enforcement also can have age progressions completed for long-term missing persons to provide updated images for public dissemination. For cases of unidentified decedents, coroners can request assistance in the collection of biological profile information, DNA sampling from bone and teeth, or dental imaging.

In its capacity as the administrator of the Repository, the LSU FACES Lab also cooperates with various state and national agencies to ensure that information on Louisiana's cold case unidentified and missing persons is represented in appropriate national databases. Working closely with the Louisiana State Police Crime Laboratory (LSPCL), FACES submits decedent and family reference DNA samples for each Repository case; DNA results then are uploaded into and can be searched at the appropriate levels of the Combined DNA Index System (CODIS). Through a partnership with the Louisiana State University Police Department, FACES can search and input unidentified persons cases into the National Crime Information Center (NCIC). Finally, all Repository cases are entered into the National Missing and Unidentified Persons System (NamUs) to allow national access to searchable case information on Repository cases.

Since the creation of the Repository, the LSU FACES Lab has seen the successful resolution of 24 long-term unidentified and missing persons cases resulting from information collected for the database. These cases include four individuals missing 5–9 years, eight missing 10–19 years, six missing 20–29 years, and six missing for more than three decades. In 13 years, the Repository has transformed from a modest database to a robust warehouse of information. Due to the nature of these cases, data collection for the Repository is never complete. Through the continued partnership between forensic anthropologists, law enforcement officers, death investigators, and other forensic science professionals, the Repository will continue to adapt to the needs of the Louisiana unidentified and missing.

#### Forensic Anthropology, Unidentified Remains, Missing Persons





### A98 Forensic Anthropology and Identification in Natural Disaster-Related Cemetery Disruptions

*Ginesse A. Listi, PhD\**, LSU Geography & Anthropology, Baton Rouge, LA 70803; *Christine L. Halling, MS*, Louisiana Department of Justice, Baton Rouge, LA 70802; *Ryan M. Seidemann, MA*, Louisiana Department of Justice, Baton Rouge, LA 70802; *Teresa V. Wilson, PhD*, Louisiana State University, Baton Rouge, LA 70803

**Learning Overview:** The goal of this presentation is to discuss the efforts undertaken to recover and identify 119 individuals displaced from their graves during a natural disaster.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing baseline information useful for recovering remains and resolving the identity of individuals displaced from cemeteries during natural disasters.

In the aftermath of natural disasters, state or national response prioritizes the living and recently deceased. Regarding the latter, forensic anthropologists often are part of a team that involves individuals from other forensic and medicolegal disciplines, and their roles range from participating with recovery to assisting in the identification process. In these cases, identification is based on typical methods used for recently deceased individuals, such as visual recognition, dental or medical records, or DNA.

When natural disasters impact the non-recently deceased (i.e., cemetery remains), identification of these individuals requires a different approach. Not only might those responsible for reintering disinterred remains have little experience with identification protocols, but also the typical methods for establishing identification are often ineffective due to the condition of the remains. In these instances, forensic anthropologists are an invaluable resource during the recovery and identification process.

Numerous natural disasters have impacted the United States over the past 15 years. In Louisiana, the most recent event was the “Great Flood” of August 2016, in which an unnamed storm dropped 19–31 inches of rain over south Louisiana within 48 hours. When the deluge stopped, an area extending more than 13,129 square miles was flooded. Local coroners were able to manage the 13 deaths that occurred during the storm; however, officials soon realized that the widespread destruction to cemeteries would require additional assistance. In total, 74 cemeteries in 19 parishes had been affected, with at least 801 graves damaged or compromised. This presentation discusses the efforts undertaken to resolve the identity of individuals displaced in the cemetery with the most damage.

Plainview Cemetery, in Livingston Parish, LA, is privately owned by a church, but had served as a burial ground for the broader community for more than 100 years. Of the 808 visible graves present prior to the flood, 250 were disrupted, with damage ranging from headstone displacement to the ejection of vaults or caskets from grave spaces. Identification for some of these individuals should have been straightforward as, since 2006, Louisiana law requires identifying information to be present on the exterior of caskets. However, only 4 of the 88 disrupted caskets that post-dated the law were in compliance. Including these 4, 12 caskets were able to be identified and reinterred without being opened. The remaining caskets had to be opened and required some level of analysis to establish the person’s identity. Anthropologists with the Louisiana Attorney General’s Office led the recovery and identification efforts, with assistance from the LA Department of Health, a disaster mortuary specialist, and anthropologists and students from the Louisiana State University Forensic Anthropology and Computer Enhancement Services (LSU FACES) Laboratory.

While the process of identification in this context mirrored forensic casework, the specialized circumstances required that additional steps be taken. Few records were available for the cemetery, so extensive post-disaster data collection was required to obtain “antemortem” comparative information. Efforts to gather data included mapping and documenting the existing graves and grave spaces and interviewing community and family members to determine who was (or had been) buried where, when, in what type of casket, and with what clothing or accessories. Recorded “postmortem” data included the biological profile and dental data for the decedent and information on antemortem trauma, personal artifacts, and the casket.

Intensive data collection was required for 119 individuals. Artifacts enclosed within the casket that bore the decedent’s name identified 60 people. All but 15 of the remaining individuals (a total of 94%) were identified by comparing the antemortem and postmortem data. In contrast to typical forensic casework, biological profile and dental data ultimately proved to be less helpful for identification, as these data were relatively homogeneous for the displaced individuals (i.e., many were elderly, edentulous, and of African ancestry). Rather, information on antemortem trauma and personal items was crucial in the identification process.

The challenges experienced at Plainview and other cemeteries, and their successful resolution, led to recently passed legislation establishing a formalized state response to disaster-related cemetery disruptions. Although each cemetery presents unique challenges, the protocols discussed here should provide a useful baseline for anthropologists in other areas when dealing with similar situations in the aftermath of natural disasters.

#### Forensic Anthropology, Natural Disasters, Identification



### A99 A Virtual Anthropology Consultancy Service (VACS)

*Lucina Hackman, PhD\*, University of Dundee, Dundee, Angus DD1 5EH, UNITED KINGDOM; Sue Black, PhD, University of Dundee, Dundee DD1 5EH, UNITED KINGDOM*

**Learning Overview:** After attending this presentation, attendees will understand: (1) the value of a VACS; (2) how the service is run by forensic anthropologists; and (3) the ways in which police forces can benefit from the use of VACS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how VACS can be developed. This presentation discusses the manners in which this forensic casework is undertaken and how it impacts and benefits the forensic anthropologists running it, as well as the police forces who access it as a resource.

In 2008, in response to repeated queries from police officers across the United Kingdom regarding bones found during building works or by gardeners, the forensic anthropologists at the Centre for Anatomy and Human Identification set up the VACS. This was designed as a triage service for all bone finds that were reported to any of the 52 police forces across all four countries of the United Kingdom. The service allowed the officers to send images of the bone/bones that had been found to a dedicated email address. The images that were received were viewed by a member of the forensic anthropology team and the reporting officer was duly contacted with an analysis of the bone or bones in the image, given advice, and provided with a report. Cases that were sent to the service were answered within an hour of receipt, seven days a week, between 7:00 a.m. and 10:00 p.m., and there was no charge to the forces for using the service. The forensic anthropologists who responded to these cases did so on a voluntary basis.

Use of the service rose rapidly, from a low of 84 cases in the first full year, increasing year by year to a high of 506 cases in 2016. The total number of cases responded to between 2008 and 2017 was 3,116. Forty-five percent of all cases were sent in the evenings and on weekends and, while percentages varied year to year, an average of 20% were human. Many of the cases were of bones found during normal activities, such as gardening, but bones were found in a huge number of situations, including during building works and renovations and by ramblers. A small number of images have been of non-osseous items, usually consisting of models of body parts, with a peak in these cases seen around the end of October, coinciding with Halloween.

Police forces incorporated the service in their Crime Scene Investigator (CSI) training and as part of Standard Operating Procedures (SOPs) for actions to take if a bone was reported. The service provided a timely answer, allowing senior officers to have a real-time response, which had an impact on their ability to decide how to deploy their officers, impacting favorably on their budget. This ability to have a quick response also meant that the service was incorporated as part of official search strategies during missing persons searches; these latter cases often resulted in images of hundreds of bones but meant that officers were not constrained waiting for specialists to arrive to confirm the origin of a bone. Each case was responded to on an individual basis and involved the production of a brief forensic report. This meant that while the forensic anthropologists running the service were not paid for the time they donated, they were able to use it as an ideal method of training students and less experienced forensic anthropologists who learn how to liaise and communicate with police, coroners, or procurators fiscal and receive instructions on how to write a forensic report. The volume of cases also provides a large repository of casework that currently acts as a teaching resource for both undergraduate and postgraduate students.

### Consultancy, Forensic Anthropology, Casework



## A100 Ancestry Estimation Using Cranial and Postcranial Macromorphoscopic Traits

Micayla C. Spiros, MS\*, Michigan State University Dept of Anthropology, East Lansing, MI 48824

**Learning Overview:** After attending this presentation, attendees will understand how combined datasets, specifically cranial and postcranial macromorphoscopic traits, can be applied to ancestry classifications.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the utility of a multiple dataset approach to ancestry estimation. Additionally, this presentation will demonstrate the importance of multivariate statistical frameworks and machine learning models to improve accuracy and validity for traditional forensic anthropological methods.

To examine the utility of combining cranial and postcranial macromorphoscopic traits, a variety of classification models were employed to estimate ancestry. The samples represent American Black ( $n=20$ ) and American White ( $n=20$ ) individuals from the Robert J. Terry Skeletal Collection and include females ( $n=21$ ) and males ( $n=19$ ) ranging in age from 24 to 80 years. Classification models using up to 8 cranial and 23 postcranial macromorphoscopic traits were performed. The classification models include: stepwise Quadratic Discriminant Analysis (QDA), artificial neural Network (aNN), Random Forest Model (RFM), and Supported Vector Machine (SVM).

Polychoric correlation was computed to assess the relationship between all variables. Overall, there was a moderately strong negative significant relationship between cranial and postcranial traits, indicating models assuming trait independence should be applied cautiously. Nasal Aperture Width (NAW) and the Left Third Trochanter (LTT), for example, were significantly negatively correlated ( $r=-0.34$ ,  $n=40$ ,  $p=0.03$ ), suggesting increases in NAW correspond to decreases in the incidence of LTT. Post-bregmatic depression was negatively correlated with all postcranial traits, except for the Left Anterior and Middle Calcaneal Facets (LAMCF) ( $r=0.225$ ,  $n=40$ ,  $p=0.04$ ), suggesting post-bregmatic depression corresponds to overall decreases in the presentation of LAMCF and increases in all other postcranial traits.

The data was divided into training and test sets, uniformly within groups using 70% of the sample for training and validation and the remainder as a hold-out test set. The results of the classification models were appealing and suggest further work is necessary. Variable importance models using the Gini index suggest both variable types are important in classification. Inferior Nasal Aperture (INA) and Spinous Process Bifurcation of the Third Cervical Vertebrae (C3SPB) were nearly equally important in all models. In fact, the stepwise QDA models using only these two variables correctly classified 90% of the sample ( $k=3$ ; AB=91%; AW=89%), a slight improvement over previous classification models utilizing a combined craniometric and cranial morphoscopic approach (AB=79.4%; AW=87.1%).<sup>1</sup> The aNN model correctly classified 95% of the holdout sample. The SVM correctly classified 97.5%, showing great promise for future work using larger sample sizes.

These models maximize classifications of ancestry by including postcranial morphology. Previous research on the utility of postcranial non-metric traits hinted at their utility in ancestry estimation methods; however, until recently, very little quantification of these traits in modern human skeletal samples has been conducted. The morphology of the postcranial skeleton is often considered too plastic to be of use in ancestry estimation; the reason may be little more than the reliance on older beliefs with no testing or verification. However, these traits show great promise in ancestry estimation, both alone and in combination with cranial macromorphoscopic traits. However, the key is robust classification models appropriate for categorical data.

### Reference(s):

- <sup>1</sup> Hefner, J.T., Spradley, M.K., Anderson, B. 2014. Ancestry Assessment Using Random Forest Modeling. *Journal of Forensic Science*. 49 (3): 583-589.

### Forensic Sciences, Forensic Anthropology, Ancestry Estimation



## A101 Macromorphoscopic Trait Analysis in Forensic Anthropological Casework

Joseph T. Hefner, PhD\*, Michigan State University, East Lansing, MI 48824; Stephen D. Ousley, PhD, Mercyhurst University, Erie, PA 16546

**Learning Overview:** After attending this presentation, attendees will be familiar with the implementation of macromorphoscopic trait data in casework associated with human identification. Attendees will learn about the development of a new analytical program for classification of ancestry and the research/analytical potential of data housed in the Macromorphoscopic Databank.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing a significant gap in best practices in the forensic anthropological approaches to macromorphoscopic trait data analysis, particularly as those data relate to the estimation of ancestry and their application in forensic anthropological casework.

Macromorphoscopic traits are primarily binary and ordinal variables of the midface and vault, generally considered skeletal framings for soft tissue variations. Large and appropriate reference samples containing Macromorphoscopic (MMS) trait data collected from individuals with modern birth years are needed for the classification models used in the estimation of ancestry from MMS traits. The Macromorphoscopic Databank (MaMD;  $N \sim 7,400$ ) serves that function, making publicly available trait scores for a large sample ( $n = 2,363$ ) of modern American populations and worldwide groups of various geographic origins ( $n = 1,790$ ). In addition, the MaMD also stores reference data for a large ( $n = 3,244$ ) sample of pre-, proto-, and historic Amerindian data, useful for biodistance studies and finer levels of analysis during Native American Graves Protection and Repatriation Act (NAGPRA) -related investigations and repatriations. In developing the MaMD, the goal was always the development of an analytical tool useful to forensic anthropological practitioners for the classification of an unknown individual into a reference population.

This presentation is intended to address the current gap in best practice concerning the implementation of MMS trait data in forensic anthropological analysis, particularly how MMS data can be successfully incorporated into the estimation of ancestry in a manner approximating craniometric analysis using the computer program *FORDISC*® 3.1. To highlight the utility of this software, this study began with known individuals (age, sex, ancestry) representing Amerindian ( $n=81$ ), Asian ( $n=74$ ), American Black ( $n=82$ ), Hispanic ( $n=82$ ), Thai ( $n=82$ ), and American White ( $n=83$ ) samples drawn randomly from the MaMD. These data were used to build and tune a number of artificial Neural Networks (aNN) using softmax modelling (decay  $< 0.001$ ) and more than 2,000 iterations. Neural networks are a form of machine learning particularly well suited for MMS data. In fact, the final model correctly classified 68% of the sample using six MMS traits. Repeated simulations ( $n=20$ ) indicate a stable classification model (CI=65.6 – 69.7;  $\bar{x}=66.8$ ;  $sd=1.16$ ). To measure any inherent bias in the model and to cross-validate the results, a second completely independent random sample was drawn from the MaMD ( $n=516$ ) and classified according to ancestry using the original model. Overall, 60% of the sample was correctly classified, ranging from 75% correct for the Asian sample to a lower percent correct for Hispanic sample (35%).

Of course, forensic anthropologists very rarely deal with samples. Instead, they are interested in an individual classification and, perhaps more importantly, measures of classification strength. Hefner and Ousley proposed Optimized Summed Score Attributes (OSSA) as a heuristic classifier using MMS traits.<sup>1</sup> OSSA has been well received but is only suitable for two groups (American Blacks and Whites) and does not provide suitable measures of classification strength. The new classification software uses several machine learning methods to best classify human remains using morphoscopic traits *drawing from the reference data contained within the MaMD*. This software permits user-selected classification algorithms and provides output similar to the results provided by *FD3*, including posterior and typicality probabilities, sensitivity, specificity, and other measures of model and classification success.

Generally, every forensic anthropological analysis includes an estimation of ancestry. Craniometric analysis traditionally relied on *FD3*, which provides statistical results to qualify conclusions and validate results through typicality and posterior probabilities. With the introduction of this software and the exploration of additional machine learning methods, MMS trait analysis can enjoy that same level of scientific rigor and method validation.

This project was supported by an award from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.

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### Macromorphoscopic Trait Analysis, Machine Learning, Optimized Summed Score Attributes



## A102 A Reevaluation of Tooth Crown Measurements in the Estimation of Ancestry Using Random Forest Classification

Michael W. Kenyhercz, PhD\*, Department of Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI 96816; Marin A. Pilloud, PhD, University of Nevada, Reno, Reno, NV 89557-0096; Joseph T. Hefner, PhD, Michigan State University, East Lansing, MI 48824

**Learning Overview:** After attending this presentation, attendees will understand the utility of tooth crown metrics in the estimation of ancestry and how random forest classification is used to achieve high classification rates among several worldwide populations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a new method in forensic anthropology and by providing a freely available Graphical User Interface (GUI) for practitioners to use in casework and research.

Teeth have long been studied for their variability among human populations, both metrically, especially using dental apportionment analysis and morphologically.<sup>1-5</sup> However, tooth crown metrics are typically outperformed by other analyses, such as craniometrics.<sup>6</sup> Pilloud et al. have recently shown the utility of tooth crown metrics in discriminating among three broad geographic groups (African, Asian, and European) using Hanihara's worldwide odontometrics dataset and linear discriminant function analysis.<sup>7</sup> When females and males of those three groups were considered separately, Pilloud et al. reported cross-validated total correct classifications of 88.1% and 71.9%, respectively, and a combined cross-validated total correct classification of 71.3%.<sup>7</sup>

Random forest classification is an alternative to linear discriminant function analysis that uses modified decision trees with non-linear boundaries. Essentially, at each decision, or tree, a classification is made and, depending on the number of trees, the group with the highest classification count is used for a classification. The user specifies the number of trees and the number of variables tested at each decision tree. For the current study, Hanihara's worldwide dataset was used with the same three broad geographic groups used in Pilloud et al.'s study.<sup>7</sup> A total of 32 tooth crown measurements are available (16 mesiodistal and 16 buccolingual), representing one side of the dental arcade. The total sample includes 1,674 complete dentitions (African Females=17, African Males=85, Asian Females=282, Asian Males=698, European Females=162, and European Males=430). The implementation of random forest classification used here includes a 70% training set, 15% validation set, and 15% testing set. Using 500 trees, with four variables tested at each decision tree, a total correct classification of the same three broad geographic groups, with females and males considered together (a total of six classification groups), achieves a total correct classification rate of 90.3%. When females and males are considered separately, the total correct classification rates are 99.1% and 98.4%, respectively.

A GUI was created with the language *shiny* in RStudio to allow practitioners to input between one and 32 variables and create custom random forest classification models on a case-by-case basis.<sup>8,9</sup> Users specify the groups to compare, which can include females and males from the three broad geographic groups: African, Asian, and European. Output includes: correct classification rates (both raw counts and percentages), total correct classification, group classification, posterior probabilities, positive and negative predictive values, individual node classification counts, out of bag error, variable importance, and a Mahalanobis distance matrix. The GUI is freely available at <https://anthropologyapps.shinyapps.io/HanihaRa/>.

### Reference(s):

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### Forensic Anthropology, Dental Crown Measurements, Random Forest Classification



### A103 Assessing the Within-Group Structure of the “Hispanic” Sample in FORDISC® 3.1

*Dorianis Mercedes Perez, MS\*, Tallahassee, FL 32303; Luis L. Cabo, MS, Mercyhurst University, Erie, PA 16546*

**Learning Overview:** After attending this presentation, attendees will better understand the limitations of ancestry estimation of “Hispanic” individuals in forensic contexts and gain insight on the misconception of utilizing the Hispanic population as a discretely defined population in typical applications of ancestry estimation methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing further research to suggest that ancestry estimation of continuously varying populations should not be employed in forensic contexts, as this may lead to greater errors in estimating biological profile parameters.

Recent decades have witnessed a significant push to improve and refine ancestry estimation methods in forensic anthropology. At the time of this writing, FORDISC® 3.1, the software for sex and ancestry estimation most commonly employed in forensic anthropology, includes a total of eight ancestries. Geographic groups characterized as Hispanic have been added in recent years, considering that a wider spectrum of representation for this generic ancestry should increase classification accuracies of unknown individuals. Thus, ancestry estimation methods have been expanding and diversifying Hispanic samples to encompass a greater range of variation. To test the effects of doing so, the within-group structure of the Hispanic sample in FORDISC® 3.1 was analyzed. Most individuals in the Hispanic reference group in FORDISC® 3.1 are of Central American origin, and the program divides them by specific country of origin (Guatemalan, Mexican, etc.). The goal of this study is to determine the validity of this reference group by using craniometric data of Hispanics from the Forensic Databank (FDB), a sample from the Dominican Republic (a Caribbean-Hispanic population), and positively identified Hispanic individuals from the New York City Office of the Chief Medical Examiner (OCME).

To assess group structure, K-means clustering analysis was performed on the pooled Hispanic sample and again on only FDB Hispanics. Analysis of the composition of clusters revealed no separation based on geographic origin in both cases. This strongly suggests that the Hispanic sample in FORDISC® 3.1 is largely an artificial group, comprised of a subjective, incomplete, and heterogeneous pool of individuals from different continental origins, rather than a real ancestry group in the traditional, geographic-origin sense. To emphasize the effects of this group composition, a modern Dominican sample was run in FORDISC® 3.1 using methods outlined in Jantz and Ousley.<sup>1</sup> Dominicans classified more frequently as American White and American Black, respectively, with only 10% of the sample classifying as Hispanic.

It is recommended that the Hispanic sample in FORDISC® 3.1 be discarded or divided and renamed as the specific geographic populations represented. Because the Hispanic population is difficult to define due to the ambiguity of characterizing the proportion of parent ancestries present on an individual basis (Native American, European, and African), it is suggested that the term “Hispanic” not be used to define an ancestral class, but an ethnicity. This would also imply that the use of craniometrics is not an appropriate approach for this type of analysis.

#### Reference(s):

<sup>1</sup> Jantz R.L., Ousley S.D. *FORDISC® 3.0: Personal Computer Forensic Discriminant Functions*. University of Tennessee, Knoxville; 2005.

#### Hispanic, Ancestry Estimation, K-Means Cluster Analysis



### A104 Testing the Homogeneity of “White”: Dental Morphology in Americans and Australians of European Descent

*Heather J.H. Edgar, PhD\*, Anthropology, Albuquerque, NM 87131; Shamsi Berry, PhD, University of Mississippi Medical Center, Jackson, MS 39216*

**Learning Overview:** After attending this presentation, attendees will understand how phenetic variation between Australians and Americans of European descent results from their specific population histories and showcases the difficulties of associating biological variation with social categories commonly used to describe missing persons.

**Impact on the Forensic Science Community:** This presentation will impact the performance of the forensic science community, especially forensic anthropology, by highlighting: (1) how dental morphological characteristics are a valuable complement to the usual tools available for estimating bioaffinity of an unknown person; and (2) how social concepts of race and ethnicity do not necessarily reflect homogeneous groups over time and space, even in populations usually considered common, well studied, and well understood.

The populations of the United States and Australia share many commonalities. Both derive first from Western Europeans, especially from Great Britain. Later migration came from other areas of Europe, including Central, Southern, and Eastern countries, especially Germany, Italy, Greece, and the Slavic nations. Still later migration to both countries included populations from Asia. However, geography and history have shaped differences in migrations to the two nations, with West Africans transplanted in large numbers to the United States and migration from Latin America being significant, while Australia continues to attract a significant number of migrants from Asia. Today and throughout its history, the greatest proportion of migrants to Australia derive from Great Britain, many via previous residence in New Zealand.<sup>1</sup>

Given that Americans and Australians of European descent, commonly known as “Whites,” both derive from migrants from the British Isles, later supplemented by Central, Southern, and Eastern Europeans, it might be assumed that there would be little to no phenetic difference between the two populations. They would be indistinguishable. In the United States, this reflects a general assumption about regional subpopulations of Whites within the nation. When forensic anthropologists use samples of European Americans in research, they are assumed to be homogeneous across regions. While many forensic anthropologists recognize that there are important differences among African Americans, Asian Americans, or especially Hispanic Americans, this recognition is not always true about European Americans. Patterned regional phenotypic variation complicates the forensic assessment of bioaffinity.

This study tested the hypothesis that Americans and Australians of European descent are phenetically homogeneous using linear discriminant function analysis. Dental morphological traits were scored following Edgar from casts of individuals born after 1980.<sup>2</sup> The samples were 63 individuals from a study of Australian twins, 100 from an orthodontic clinic in Memphis, TN, and 34 from an orthodontic clinic in New York City, NY. Data consisted of 25 dental morphological traits that could be scored and varied significantly between Australian samples and the combined United States. Correct allocation between the Australian and combined United States sample was 69.1%. Comparing the New York and Australian samples, 66.7% were correctly allocated, while Tennessee versus Australian samples were correctly identified 69.1% of the time. A comparison of the Tennessee and New York samples yielded a marginally better result, with 75.8% correct allocation.

These results may indicate that the variation in Australians of European descent is subsumed by that in Americans of European descent. The fact that Australian Whites could be discerned from American Whites at a rate significantly different from chance indicates that there is significant variation between the two populations. Interestingly, the highest rate of correct classification was between the two American samples, indicating the presence of regional heterogeneity within Americans of European descent. This patterned heterogeneity warrants further investigation, as estimates of bioaffinity are commonly applied nationwide that have been derived solely from regionally isolated populations.

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- <sup>2</sup> Edgar H.J.H. 2017. *Dental Morphology for Anthropology: An Illustrated Manual*. London: Routledge.

#### European American, European Australian, Dental Morphology



### A105 Estimating Ancestry and Region of Origin: A Multidisciplinary Approach to the Identification of Unknown Individuals

Angela Soler, PhD\*, NYC Office of Chief Medical Examiner, New York, NY 10016; Andrew J. Schweighardt, PhD, Northport, NY 11768; Laura Huynh, MS, NYC Office of Chief Medical Examiner, New York, NY 10016; Carl Gajewski, MS, Office of Chief Medical Examiner, New York, NY 10016

**Learning Overview:** After attending this presentation, attendees will better understand the benefits and limitations of using free, publicly available mitochondrial DNA (mtDNA) databases to complement visual and anthropological ancestry estimates in cases of unidentified individuals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how mtDNA ancestry predictions perform in comparison to visual and anthropological ancestry estimates. In addition, the mtDNA databases will be assessed for their utility in making predictions on region of origin, which could be especially useful in the identification of migrants.

Forensic anthropologists often collaborate with other specialties to assist in the identification of unknown individuals by contributing biological profiles, as well as by providing investigative resources, maintaining oversight of long-term unidentified individuals, and by suggesting innovative scientific approaches. The purpose of this study is: (1) to determine the accuracy of ancestry estimates from mtDNA databases and assess their utility in building upon ancestry estimates (both anthropological and visual), and (2) to determine their efficacy in estimating an individual's region of origin, especially in individuals who are potentially migrants. This research also highlights the benefits of collaboration between the forensic science disciplines, as demonstrated by the Forensic Anthropology and Forensic Biology Units at the New York City Office of Chief Medical Examiner (OCME).

The test sample consists of 41 OCME cases of identified (20) and unidentified (21) individuals for whom an mtDNA profile was developed. The sample consists of 19 cases in which ancestry was visually assessed and 22 cases in which ancestry was anthropologically estimated using macromorphoscopic traits and craniometrics. An additional 11 OCME staff members were included for quality control measures.

The mitochondrial sequences of each individual were searched through the European DNA Profiling Group (EDNAP) mtDNA Population Database (EMPOP) mtDNA online database, v3/R11. The haplotype lineages and region of origin predictions produced by EMPOP were then compared to the ancestry estimates. All mtDNA sequences and anthropological ancestry estimates were generated by the Forensic Biology and Forensic Anthropology Units at the OCME.

Results revealed 83% (34/41) overall agreement between the haplotypes derived from the EMPOP database and the visual and anthropological estimates of OCME cases. Comparison of the mtDNA results with visually assessed ancestry revealed 89% agreement (17/19), while comparison with anthropologically derived ancestry estimates revealed 77% agreement (17/22). Of the anthropology cases in which the two methods yielded conflicting ancestral predictions, approximately 40% exhibited incomplete/damaged crania and 40% exhibited morphoscopic traits/craniometrics consistent with multiple ancestral groups. For all known/identified individuals, 6 of 7 anthropological ancestry estimates and 19 of 20 haplotype predictions overlapped with the individual's confirmed ancestry. Individuals of confirmed "Hispanic" and "Middle Eastern" ancestry may exhibit either African, Asian, or European haplotypes and, therefore, any of these haplotypes would be considered overlapping with a "Hispanic" or "Middle Eastern" visual/anthropological assessment. An additional 11 OCME staff members were tested for quality control measures, and 9 of these individuals exhibited haplotypes that matched their confirmed ancestry.

The OCME dataset includes 8 individuals with known region of origin, which was confirmed through discussions with families and foreign consulates during the identification process. The haplotype density maps produced by EMPOP overlapped with the region of origin in 6 of the 8 cases. A quality control test of 11 OCME staff members demonstrated overlap in regions highlighted on the map in only 6 individuals. Maps for individuals with European and African maternal lineages were diffuse and non-specific, while those of Asian maternal lineages, AfroCaribbean, as well as "Hispanic" lineages from Central and South America were predicted with greater specificity.

Results indicate that mtDNA haplotype predictions could be a useful complement to the anthropological or visual ancestry assessment. These databases are free and publicly available and, with the collaboration of forensic biology and anthropology experts, could provide additional investigative leads. However, since mtDNA only reflects the maternal lineage and ancestral self-identification is complex, it is not recommended to use these databases without collaboration with anthropologists and biologists. In certain cases, the EMPOP database density maps can add additional information that is informative to the identification of potential migrants, especially those of Asian or Central and South American origins. Preliminary results indicate that Y-chromosomal Short Tandem Repeat (Y-STR) databases also show promise in predicting ancestry and region of origin for unidentified male individuals.

#### Ancestry Estimation, Mitochondrial DNA Ancestry Database, Unidentified Remains





### A106 Using Mitochondrial DNA (mtDNA) -Derived Maternal Ancestry to Assist in Forensic Anthropology Investigations of Deceased Unidentified Individuals From the United States-Mexico Border

Cris E. Hughes, PhD\*, Department of Anthropology, Urbana, IL 61801; Charla Marshall, PhD, Armed Forces DNA Identification Laboratory, Dover Air Force Base, DE 19902; Sonia E. John, University of Illinois at Urbana-Champaign, Urbana, IL 61801; Bruce E. Anderson, PhD, PCOME, Tucson, AZ 85714

**Learning Overview:** The goal of this presentation is to demonstrate how mtDNA can be particularly useful for counties proximate to points of migrant entry along the United States-Mexico border, where the preliminary questions asked by investigators include whether the individual is an Undocumented Border Crosser (UBC) and an American citizen.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how forensic anthropologists and investigators can apply mtDNA in an alternative manner to both produce useful investigative leads and compare forensic anthropological estimates of ancestry.

MtDNA is commonly used in forensic cases of degraded DNA, such as at the Armed Forces DNA Identification Laboratory and Pima County Office of the Medical Examiner (PCOME), where unidentified individuals have been exposed to harsh or temporally elongated taphonomic processes. Because mtDNA is non-recombining, it is primarily used for exclusions of family reference samples in casework, as the sequence variants are not unique to a person but shared among maternally related individuals. Closely related mtDNA lineages with a shared evolutionary history are grouped into mitochondrial haplogroups. The geographic structuring of mtDNA haplogroups entails that certain haplogroups are in higher frequencies in specific areas than others. Population genetic studies have demonstrated a strong relationship between continental geography and mitochondrial haplogroups, such that an individual's haplogroup assignment can often be a proxy for maternal biogeographic ancestry.

Thus, mtDNA can be useful in some cases as an investigative lead by providing information about maternal ancestry.<sup>1</sup> The object of this presentation is to demonstrate how mtDNA can be particularly useful for counties proximate to points of migrant entry along the United States-Mexico border, where the preliminary questions asked by investigators include whether the individual is a UBC and whether they are an American citizen. UBCs are typically of Mesoamerican and Central American origin, with UBCs from the Caribbean and South America less common. More rarely, the border is a point of entry by individuals with ties to distant locations, such as the Middle East.<sup>2</sup> Current forensic anthropological ancestry estimation methods from the skeleton emphasize the vast range of cranial variation exhibited by Latino populations; thus, it is often difficult to accurately estimate ancestry from the skeleton alone, and having an additional data point such as maternal ancestry could produce a more definitive investigative lead.<sup>3-6</sup>

On average, Latino populations from common migrant-sending nations overwhelmingly exhibit mtDNA haplogroups of Native American origin, with approximately 2%–10% exhibiting haplogroups not native to the Americas.<sup>7-10</sup> By understanding the population histories and mtDNA variation of source populations (both common and uncommon) of UBCs, mtDNA haplogroups can assist investigators with their approach to an investigation. In this presentation, attendees will learn how mtDNA can assist an investigation in two ways: differentiating UBC from non-UBC cases and differentiating whether an individual is from a traditional or potentially non-traditional sending region. If hypothesizing that the majority of UBCs will have haplogroups native to the Americas, then those individual cases whose mtDNA haplogroups are non-native should be flagged for consideration as a non-UBC or non-traditional UBC case.

This study includes data on 340 cases from PCOME. Haplogroup calls were made using the European DNA Profiling Group mtDNA Population Database (EMPOP) and confirmed by a qualified analyst. Based on known haplogroup frequencies of Latino populations, the hypothesis that approximately 90%–98% of the PCOME UBC cohort will exhibit haplogroups native to the Americas was tested.<sup>7-10</sup> Forty-seven cases exhibit non-native haplogroups, the majority of which ( $n=36$ ) are believed to be or identified as UBCs from Latin America. Therefore, approximately 10% of the entire PCOME sample exhibits non-native Eurasian haplogroups, supporting the hypothesis. This study also explored the proposed pipeline of flagging cases exhibiting non-native haplogroups as potential non-UBC or non-traditional UBC cases. Eleven of the non-native haplogroups belonged to cases non-UBCs and were all identified as or believed to be White American citizens. Five of the non-native cases had haplogroups of African maternal ancestry, and most of these were classified as UBCs, with forensic anthropological estimates of ancestry highlighting African or unsure ancestry. The cases with non-native haplogroups believed to be/identified as UBCs were not unexpected, as most of the haplogroups associated with these cases were documented in Spanish and/or Hispanic reference population samples by EMPOP; thus, regardless of the non-native haplogroup status, they are consistent with Latino population history, including Spanish gene flow. The study results provided in this presentation demonstrate how forensic anthropologists and investigators can apply mtDNA in an alternative manner to produce useful investigative leads.

*The opinions and assertions presented hereafter are the private views of the authors and should not be construed as official or as reflecting the views of the United States government.*

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### United States-Mexico Border, Ancestry, Mitochondrial DNA



## A107 New Methods for Forensic Age Estimation Based on Dental Development

Kelly Heim, PhD\*, St. Petersburg, FL 33714; Marin A. Pilloud, PhD, University of Nevada, Reno, Reno, NV 89557-0096; Hassem Geha, DDS, University of Texas Health Science Center, San Antonio, TX 78229; Ying Wu, DDS, PhD, Oregon Health Science University, Portland, OR 97201

**Learning Overview:** After attending this presentation, attendees will understand the role of population variation in dental development and its impact on age estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting data on a large set of radiographs exploring dental development in a diverse group within the United States. This research examines the role of various biological parameters on dental development and presents new methods for age estimation in a forensic context in the United States.

Dental development is considered the most accurate method of age estimation in subadults because this process is less subject to external and internal influences than skeletal development. However, research has suggested that dental development can be affected by secular change, sex, and ancestry.<sup>1-6</sup> Therefore, it is problematic that the established methods for subadult dental age estimation in the United States do not accurately represent the population to which they are applied. This project proposes new methods for age estimation based on a large, modern sample of United States children representing various ancestry groups.

Using the scoring system developed by Demirjian et al., dental development was evaluated from 1,757 orthopantomograms of individuals ages 5–20 years taken between 1972 and 2017.<sup>7</sup> Orthopantomograms were obtained from three sources: the James K. Economides Orthodontics Case File System from the Maxwell Museum of Anthropology at the University of New Mexico; the School of Dentistry at the University of Texas Health Science Center at San Antonio; and the Oregon Health Sciences University School of Dentistry. Age estimation methods that did and did not account for sex and/or ancestry differences were created from a training subset of the total sample. Confidence Intervals (CIs) were created for each developmental score for every tooth in the training sample. Additionally, linear models were created to estimate age from multiple teeth.

Overall, dental development among females is advanced compared to males, except in the third molar. Results also indicate that Hispanic individuals tend to develop teeth more quickly when compared to European Americans. However, CIs based on all individuals are comparably accurate to, and more precise than, CIs based on group-specific subsets (i.e., CIs divided by sex and ancestry). Further, linear models based on all individuals also exhibit comparable accuracy and precision to those based on subsets divided by sex and ancestry. The group-specific models with the highest performance measures, those based on females, males, European Americans, and Hispanics, are slightly more accurate and precise than models based on all individuals and are therefore recommended when these parameters are known.

The methods presented here fill a void in the field, as this work represents the only current age estimation method using dental development derived from a modern American population. Results suggest that general methods can be used without losing precision and accuracy, which is helpful in a forensic context as it can be difficult to estimate sex and ancestry from juvenile skeletal remains.

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### Age Estimation, Dental Development, Population Variation



### **A108 Variations in Skeletal and Dental Growth and Development Patterns and Their Effect on Age Estimation: A Preliminary Study of Five Populations**

*Louise K. Corron, PhD\**, University of Nevada, Reno, Anthropology Dept, Reno, NV 89557; *Kyra E. Stull, PhD*, University of Nevada, Reno, Reno, NV 89557; *Michael H. Price, PhD*, Santa Fe Institute, Santa Fe, NM 87501; *Yueran Yang, PhD*, University of Nevada, Reno, Psychology Dept, Reno, NV 89557

**Learning Overview:** After attending this presentation, attendees will understand the effects of genetic and environmental factors on skeletal and dental growth and development patterns and if these factors can affect resulting age estimates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by participating in the debate on global versus population-specific methods for subadult age estimation and by increasing knowledge regarding the effects of genetics and the environment on age estimates.

Skeletal and dental indicators of growth and development, such as bone measurements, epiphyseal fusion stages, and dental development stages, are widely used for subadult age estimation in forensic anthropology. However, these age indicators can present with different patterns among different groups, as they are influenced by various genetic and environmental factors. Consequently, children of the same age from different populations may present different values for these indicators, which can lead to misinterpretations of age, sometimes with serious legal consequences. Because variation increases with age, the risk of error increases with age, especially from late childhood onward. This study seeks to qualify the differences in skeletal and dental growth and development patterns between five different populations characterized by different origins and Socio-Economic Status (SES) and evaluate the impact of these differences on age estimation.

Diaphyseal and pelvic dimensions, epiphyseal fusion stages, and development stages of the permanent dentition were collected from approximately 2,000 contemporary children between birth and the age of 15 years. Two countries, Colombia and South Africa, are considered to be of low SES (i.e., high Gini Coefficient), and three countries, France, the Netherlands, and the United States, are considered to be of high SES (i.e., low Gini Coefficient). Differences between growth and development patterns were assessed using simple visualizations (boxplots and scatterplots) for each indicator and population. A hierarchical modeling approach was employed to account for variability at different levels (i.e., within-country and between-country). The Hierarchical Linear Model (HLM) enables us to examine whether different environments (different countries in the data) would influence the relationship between age and the indicators. Age estimates were derived from a Bayesian mixed probit model. Models were built for all populations pooled and separated according to three life history age groups: birth to 3 years, 3 to 7 years, and 7 to 12 years. Accuracy was measured as the difference between estimates and true chronological age.

Diaphyseal growth shows a similar growth pattern in all groups, but the HLM models identified differences among the populations. Boxplots of dental development and age show country-level differences; children from higher SES countries present with earlier dental development than Colombian or South African children. HLM models corroborate the visual comparison; each country presents with a unique slope and intercept. These differences seem to point to a population-specific approach rather than a universal approach for age estimation. While growth and development indicators were statistically different in the HLM model, the resulting 95% confidence intervals generated for each country-specific Bayesian mixed probit model overlapped and the pooled sample presents with low overall root mean squared error values. Based on these findings, it seems possible to use a global approach. However, the reference samples chosen to inform the model should ideally be similar in population history and economic status.

Indicators with the highest predictive power retained in the life-history models mirror the ongoing sequence of growth and development activity in accordance with age. These indicators are femoral, tibial, and humeral diaphyseal lengths and first mandibular molar stages during the first life history stage; femoral diaphyseal length and second maxillary molar stages during the second life history stage; and femoral diaphyseal length during the third life history stage and in the model with all ages combined. This study demonstrates that accurate age estimates can be obtained for subadults using multivariable models based on skeletal and dental data, independent of their population of origin.

#### **Growth Patterns, Mixed Probit, Socio-Economic Status**



### A109 Estimation of Juvenile Age at Death Using Anterior-Posterior (AP) Radiographs of the Knee

Rachel E. Smith\*, Marquette, MI 49855; Carolyn V. Isaac, PhD, Kalamazoo, MI 49008-8074; Jered B. Cornelison, PhD, Western Michigan University School of Medicine, Kalamazoo, MI 49008; Jane Wankmiller, PhD, Northern Michigan University, Marquette, MI 49855

**Learning Overview:** After attending this presentation, attendees will be familiar with a new measurement-based method of age-at-death estimation for juvenile decedents using radiographs of the lower limb.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new metric juvenile age determination method that highlights the value of using the metaphyseal regions of long bones for age determination. This method is applicable for complete remains and remains that may be incomplete due to decomposition, trauma, or dismemberment, and offers the potential to narrow current age range estimates, particularly for young juveniles.

Except in cases of extreme malnourishment or other biological stressors, bone growth and development in juveniles generally occurs at relatively predictable rates.<sup>1</sup> Building upon this predictability, several reliable age determination methods exist, including but not limited to those based on dental development, epiphyseal appearance and fusion, and the diaphyseal length of many long bones. The timing of the appearance of primary and secondary ossification centers and their fusion provides forensic anthropologists with fairly narrow age ranges for juveniles; however, these methods do not take into account the individual development of the epiphyses and metaphyses and their relationships to one another. During juvenile development of long bones, given that the metaphysis is present well before the appearance of the secondary ossification center of the epiphysis, the epiphysis must undergo a rapid widening to match the width of the metaphysis before the two elements approximate each other's size and later fuse together. This observed difference in the metaphyseal and epiphyseal growth rates raises the question of predictability in the metaphysis-to-epiphysis size ratio—can this ratio be used to reliably estimate age? Cunningham et al. report that the distal femoral epiphysis approximates the width of the metaphysis by age 7 in girls and age 9 in boys, suggesting the metaphyseal region to be a useful tool in juvenile age estimation.<sup>2</sup>

This study explores the relationships between the development of the distal femoral and proximal tibial metaphyses and their respective epiphyses. Anterior-posterior radiographs of the lower limbs of 111 juveniles of known age (61 males, 50 females; ages 0 to 18 years) are included in this study. Measurements of the femoral and tibial metaphyses and epiphyses were documented using the Picture Archiving and Communication System (PACS) at the Western Michigan University Homer Stryker M.D. School of Medicine, Department of Pathology (WMed). Measurements of the metaphyses and epiphyses associated with the knee were used to generate ratios for each individual, which were then used to establish ratio-based age cohorts.

Results from preliminary statistical analyses suggest that the distal femur and proximal tibia metaphysis/epiphysis diameter ratios may be reliable for determining a relatively narrow age range for young juveniles. Differential development of the femur and tibia must be considered when applying this method, as their respective ratios for different age cohorts vary. This presentation will discuss inter- and intra-observer error rates associated with this method, as well as the manners in which juveniles' circumstances of life and death affect its applicability and efficacy.<sup>3</sup> Overall, this method is reliable and repeatable and may provide forensic anthropologists with an additional tool for juvenile age determination, which is especially applicable in situations when remains do not involve complete long bones and/or dentition.

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#### Radiography, Age-at-Death Estimation, Knee



**A110 WITHDRAWN**

### A111 Staging Clavicular Development on Magnetic Resonance Imaging (MRI): Pitfalls and Recommendations for Age Estimation

Jannick De Tobel, MD\*, Ghent University, Department of Radiology, Ghent, Oost-Vlaanderen 9000, BELGIUM; Elke Hillewig, MSc, Universiteit Gent, Vakgroep Radiologie, Gent 9000, BELGIUM; Mayonne Van Wijk, MSc, Netherlands Forensic Institute, The Hague 2497 GB, NETHERLANDS; Steffen Fieuws, Leuven, VB, BELGIUM; Patrick W. Thevissen, PhD, KU Leuven, Leuven, Vlaams-Brabant B-3000, BELGIUM; Koenraad L. Verstraete, PhD, Universiteit Gent, Vakgroep Radiologie, Gent 9000, BELGIUM

**Learning Overview:** After attending this presentation, attendees will be aware of the pitfalls of staging clavicular development on Magnetic Resonance Imaging (MRI) for age estimation and how to take those pitfalls into account to estimate age in a proper manner.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by elaborating on the emerging field of radiation-free age estimation by means of MRI. It will be demonstrated that even experienced researchers are prone to the pitfalls of assessing clavicle MRI, which affects stage allocation for age estimation.

**Background:** In age estimation, a particularly challenging region of interest is the sternal end of the clavicle, which is studied when the age threshold of 18 years is of importance. Few studies have applied MRI of this region in the living, which may be due to the prevalence of motion artifacts and shape variants, impeding an assessment of clavicular development.<sup>1-5</sup>

Regarding early and late clavicular development, two ambiguities have been pointed out in the literature. First, Hillewig et al. highlighted that the physal scar was hard to discern on MRI.<sup>1</sup> This was confirmed in a larger study sample, while the physal scar had not disappeared in any of Schmidt et al.'s participants.<sup>2,3</sup> Second, the fully mature stage resembles the earliest stage, in which the epiphysis has not yet begun mineralization. Therefore, those two stages may be confused and wrongfully allocated.<sup>2</sup> It should be noted that this problem was not encountered by Schmidt et al.; they stated that discerning early from late clavicular development should be based solely on the sternal end's shape, not on the hand/wrist status.<sup>3</sup>

Regarding physal bridging, substages proved to be useful in determining whether the age thresholds of 18 and 21 years old had been reached.<sup>3,5</sup> However, none of these studies reported the corresponding probabilities to having reached those age thresholds associated with the different substages.

**Purpose:** To elaborate on these pitfalls and this staging technique and to propose recommendations for a proper use of clavicle MRI for forensic age estimation.

**Materials and Methods:** Three Tesla MRIs were conducted on both clavicles in 524 healthy Caucasian volunteers (277 females, 247 males) between 14 and 30 years of age. Two observers assessed the images independently, applying the most elaborate staging technique that has been described in the literature.<sup>6,7</sup> Consequently, a Bayesian model for age estimation used the data and its performance was tested.<sup>8</sup> Two aspects of age estimation were studied using the model: (1) point prediction of age and its uncertainty; and (2) the ability to discern minors from adults.

**Hypotheses:** (1) early (stage 1) and late development (stages 4 and 5) of the clavicle's sternal end should be disregarded for age estimation; (2) substages before bridging of the physal plate has started (stage 2) disturb the chronology of stages. Therefore, they should be considered jointly as one stage and incorporated accordingly into the model for age estimation; and (3) conversely, substages of the bridging physal plate (stage 3) can successfully be incorporated into a Bayesian model for age estimation, rendering acceptable point predictions of age and diagnostic indices to discern minors from adults.

**Preliminary Results:** Weighted kappas of 0.77 for intra-observer agreement and 0.64 for inter-observer agreement indicated large discrepancies between observations. These were mainly caused by interchangeably allocating stage 1 and stage 4 or 5. When one observer allocated stage 1, the other observer allocated stage 4 or 5 in 12% (3/26) of cases; vice versa occurred in 30% (16/53) of cases. This confirmed hypothesis 1.

Considering age distributions within substages of stages 2 and 3, the chronology was disturbed, since bridging did not wait until the epiphysis had widened. Moreover, mean ages within substages of stage 2 did not increase consecutively. This confirmed hypothesis 2. By contrast, the mean ages in substages of stage 3 did increase consecutively, even when substages of stage 3a were considered. This confirmed hypothesis 3.

Performance of the Bayesian model for age estimation was not yet assessed at the time this presentation was submitted.

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### **Age Estimation, Clavicle, Magnetic Resonance Imaging**





### A112 Age-at-Death Estimation: Accuracy and Reliability of Age-Reporting Strategies

Christine Bailey, MA\*, Western Carolina University, Cullowhee, NC 28723

**Learning Overview:** After attending this presentation, attendees will better understand the performance of different age-reporting strategies that can be used to report a final age estimation to law enforcement agencies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insights regarding the efficacy of common aging methods and the different strategies relied upon to develop a final age range for unidentified human remains.

Age-at-death estimations are an informative aspect of biological profiles as they help law enforcement to narrow potential victim identifications. However, age-at-death estimation continues to be a challenge within forensic anthropology due to the uncertainty regarding method selection and the production of a final estimation for law enforcement.

The purpose of this research is to identify the age-reporting strategies that provide the most accurate and reliable (low inaccuracy and low bias) age-at-death estimations when evaluated by total sample, age-cohort (20–39; 40–59; 60–79), and sex. The age-reporting strategies in this study were derived from six age-at-death estimation methods and tested on 58 adult individuals (31 males and 27 females) from the William M. Bass Donated Skeletal Collection at the University of Tennessee, Knoxville. An experience-based approach in which the observer produced a final estimation using the data collected and their expert judgement was included to assess the appropriateness of experience-based estimations in medicolegal contexts.

The results demonstrate that the most accurate and reliable age-reporting strategy varied if the sample was evaluated as a whole, by age, or by sex. The most accurate and reliable strategy for the total sample was the experience-based approach (accuracy=80%, inaccuracy=6.1 years, bias=-0.6 years). When the sample was divided by age, Suchey-Brooks pubic symphysis performed the best for the 20–39 age-cohort (accuracy=89%, inaccuracy=8.8 years, bias=0.5 years), the experience-based approach for the 40–59 age-cohort (accuracy=85%, inaccuracy=5.6 years, bias=-0.1 years), and Buckberry-Chamberlain auricular surface for the 60–79 age-cohort (accuracy=100%, inaccuracy=7.9 years, bias=-5.3 years). Finally, when separated by sex, Hartnett pubic symphysis performed the best for males (accuracy=81%, inaccuracy=10.6 years, bias=-1.0 years), and the experienced-based approach performed the best for females (accuracy=89%, inaccuracy=4.9 years, bias=-0.4 years).

While none of the age-reporting strategies evaluated in this study were consistently the most accurate and reliable for all the sample categories, the experience-based approach performed well in each category. This research helps shed light on the performance of different age-reporting strategies and provides further support to the reliance on multiple aging indicators and professional judgment in developing a final age-at-death estimation.

#### Age-at-Death Estimation, Age-Reporting Strategies, Biological Profile



### A113 The Validation of a New Age-at-Death Method Utilizing Osteoarthritis (OA) of the Shoulder

Morgan J. Ferrell\*, University of Central Florida, Orlando, FL 32817; Jonathan D. Bethard, PhD\*, University of South Florida, Tampa, FL 33620-8100

**Learning Overview:** After attending this presentation, attendees will understand how OA of the shoulder may provide useful information for age-at-death estimation in forensic anthropological contexts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that forensic anthropologists may be able to utilize degenerative changes in the shoulder girdle for estimating age at death.

One of the primary challenges faced by forensic anthropologists and bioarchaeologists is generating age-at-death estimates. Recently, Brennaman et al. developed a method for adult age-at-death estimation which utilizes OA of the shoulder.<sup>1</sup> When new age-at-death estimation techniques are developed, it is necessary for other researchers to test the methods; therefore, this study is a validation of the Brennaman et al. methodology. The final sample was drawn from two skeletal collections: the William M. Bass Donated Skeletal Collection ( $n=129$ ) and the 18<sup>th</sup>-19<sup>th</sup>-century documented skeletal collection of St. Bride's Church ( $n=60$ ). Following the method developed by Brennaman et al., four joint surfaces were examined for osteoarthritis and scored: (1) the glenoid fossa of the scapula; (2) the head of the humerus; (3) the acromial facet of the scapula; and (4) the lateral facet of the clavicle.<sup>1</sup> The ordinal scoring system was used to assess and document the prevalence of lipping, surface porosity, osteophyte formation, eburnation, and the estimated percentage of the bone surface area affected by each OA feature. The ordinal scores were tabulated to create composite scores and subsequently utilized to generate age-at-death estimates. Age-at-death estimates were generated using Brennaman et al. and were compared to individuals' real ages to understand the method's bias and inaccuracy.<sup>1</sup>

High inaccuracy scores (right shoulder=28.39; left shoulder=26.36) indicate that the method consistently did not generate accurate age estimates, with most ages underestimated. The scores for the acromioclavicular joint were frequently higher than the scores for the glenohumeral joint. Therefore, to assess whether the acromioclavicular joint surfaces were having a greater impact on the overall composite scores, a paired *t*-test was performed. The paired *t*-test results indicate that the lateral facet of the clavicle and the acromial facet had the greatest impact on overall composite scores in both the Bass Collection and St. Bride's Collection samples. Overall, the results from this study suggest that OA of the acromioclavicular joint may be a stronger indicator of age than OA of the glenohumeral joint.

There were several problems noted during the present research: (1) while numerous studies have shown that there is a correlation between OA and age, the variability in its presentation and prevalence was apparent in this study; (2) porosity was not common on the glenoid fossa and humeral head, and eburnation scores were low for all joint surfaces, except for a few severe cases; and (3) this method requires all four joint surfaces to be present, which will not always be the case in forensic and archaeological contexts. Additionally, future research of OA on the acromioclavicular joint surfaces may lead to the development of a new method of age-at-death estimation utilizing only this joint complex.

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#### Age-At-Death, Osteoarthritis, Shoulder



### A114 The Application of Dental Cementochronology in Unidentified Migrants in South Texas

Mary C. Swearingen, MA\*, Texas State University, San Marcos, San Marcos, TX 78666; Nicholas P. Herrmann, PhD, Texas State University, San Marcos, TX 78666; Kate Spradley, PhD, Texas State University, San Marcos, TX 78666; Sophia Mavroudas, MA, Texas State University, San Marcos, TX 78666

**Learning Overview:** After attending this presentation, attendees will understand the correlation of histological and skeletal morphological methods used for estimating age at death of Latin American migrants along the United States-Mexico border.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an additional aging technique for Latin American migrants when macroscopic and rib histological aging techniques are not possible.

The number of reported deaths of undocumented border crossers along the United States-Mexico border has been steadily increasing despite a decrease in apprehensions since the early 1990s.<sup>1</sup> This is due to the “Prevention Through Deterrence” policy, implemented by the Clinton administration and enforced by the United States Border Patrol.<sup>1</sup> This policy has inadvertently pressured undocumented migrants away from urban communities and into the hostile desert environment in which many individuals succumb to exposure to dehydration, heat, and hunger. The humanitarian effort to put names to the unidentified migrant remains that are found in Texas is referred to as Operation Identification (OpID). A total of 279 individuals have been transferred to Texas State University for anthropological analysis.

One of the critical factors to consider when comparing potential identification matches between missing persons reports and forensic anthropology reports, is an accurate age-at-death estimate. Previous studies have examined the accuracy of macromorphoscopic aging techniques based on the Anthropological Database at Odense University (ADBOU) scoring procedures and rib histological analysis.<sup>2</sup> The goal of the current study is to examine the utility of cementochronology as an additional aging technique for Latin American migrants when ribs or ossa coxae are not preserved. Additionally, the outermost annulation of each tooth section was used to estimate season of death based on recommendations suggested by Dr. Wedel.<sup>3</sup>

One single rooted tooth was sampled from 48 individuals from the OpID cases (18 females and 30 males). Thirty of the individuals included in this study had rib histological aging analysis completed. The rib histology ages were gathered from case reports. The cementochronology age estimates were compared to rib histology and ADBOU skeletal age estimates. Point age estimates were used to test the correlation among the three methods. A Wilcoxon rank signed test was used to test the consistency between annulation counts among the three observations. This test showed that the annulation counts per individual were consistent throughout all the observations (90% of the counts had a count difference of 2 or less). For 9 of the 48 cases, the known season of death was established based on Accumulated Degree Days (ADD), which were then compared to the estimated season of death based on the outermost annulation of the dental cementum.

Results indicate that the rib histology, ADBOU, and cementochronology aging methods had statistically significant positive correlations based on scatterplot matrices based on the Kendall’s Tau correlation coefficients ( $p < 0.05$ ). These results indicate that cementochronology point age estimates were consistent with the rib histology point age estimates. There have been two positive identifications of individuals that were included in this sample. The cementochronology point age estimates were consistent with the known age, whereas the ADBOU ages tended to overage young individuals and underage older individuals. The rib histology age ranges encompassed the known age of the two identified individuals; however, this age range is rather large and was likely to capture the known age. The cementochronology age ranges for the two identified individuals were narrower. The estimated season of death for six of the nine cases matched the known season of death. Once more positive identifications are made, the accuracy of season of death from cementochronology can be further investigated. Additionally, the correlation of age estimates derived from ADBOU, rib histology, and cementochronology can be examined for more individuals as more identifications occur.

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#### Dental Histology, Aging, Operation Identification



### A115 Ancestral Variation of the Relative Proportionality of Skeletal Facial Features: A Metric Assessment

Janet E. Finlayson, MA\*, University of Florida, Gainesville, FL 32610; Michala K. Stock, PhD, High Point University, High Point, NC 27268

**Learning Overview:** The goal of this presentation is to assess and describe the relative proportionality of skeletal facial features and their variation by ancestral origin. After attending this presentation, attendees will understand the covariance of select craniofacial attributes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing empirical, metrically based observations of how facial features covary with each other, developing a basis to define suites of meaningful traits for skeletal analyses. The results of this study can inform the evaluation of macromorphoscopic traits when assessing a skeletal individual's ancestral affiliation.

Standard non-metric approaches to skeletal ancestry assessment rely on analyses of the shape or size of individual facial features, which are sometimes considered in isolation and sometimes in relation to other traits or to the somewhat vague “facial skeleton” as a whole. This presentation seeks to better define the proportional relationships between various facial features that are commonly used in non-metric sex assessment and to test the hypothesis of positive scaling between overall facial dimensions and the dimensions of pertinent facial features.

This study accessed 3D cranial scans and demographic data of 27 (Black=8; Hispanic=3; White=16) identified individuals analyzed at the University of Florida C.A. Pound Human Identification Laboratory between 1989 and 2018. Selection of individuals included the following criteria: (1) 3D cranial surface scans, collected using a NextEngine™ 3D Laser Scanner and ScanStudio™ software, were available; (2) an age of at least 18 years at death; and (3) select craniofacial landmarks were not affected by pathological conditions, trauma, postmortem damage, or anomalous variation. Coordinate data of 14 craniofacial landmarks were collected for each individual and analyzed in R™.<sup>1</sup> From these data, Inter-Landmark Distances (ILDs) were calculated for eight craniofacial dimensions representing widths or heights of various facial features: nasal height; nasal aperture height; nasal aperture width; facial height; upper, middle, and lower facial widths; and interorbital breadth. Linear model regression analyses were then performed to evaluate ILD relationships.

A factorial Multivariate Analysis of Variance (MANOVA) demonstrated that while the main effects of sex and ancestry were both significant ( $p=0.017$  and  $p=0.039$ , respectively), their interaction was not; thus, sexes were pooled for further analyses. Results indicate weak-to-moderate ( $R^2$  values range between 0.24 and 0.39) but significant ( $\alpha=0.05$ ) correlations between all width/breadth ILD pairings, except the comparison of interorbital breadth to lower facial width. This supports that facial features in anatomical proximity to each other (i.e., their development is linked, or they undergo similar biomechanical stresses) are more likely to have meaningful covariation, suggesting that suites of certain traits and their relative proportionalities should be considered in ancestry assessments. Unsurprisingly, given the proximity of nasospinale and prosthion, nasal height is significantly correlated with total facial height in the pooled sample ( $R^2=0.43$ ,  $p=0.0002$ ); however, correlations between nasal aperture height/facial height and nasal aperture height/nasal height reach significance only for the White group.

There is a discernable pattern to the sorting of the Black and White groups in the majority of these comparisons of facial proportionality, with minimal to moderate overlap of these groups. The Hispanic individuals did not sort into any discernible group or pattern, instead generally falling into intermediate morphospace, likely resulting from both their low sample size and issues with using “Hispanic” as a category for ancestral affiliation. The results reveal several expected outcomes, including that Black individuals have absolutely wider nasal apertures and White individuals express absolutely taller nasal apertures, resulting in the nasal proportions typically associated with these two groups. Conversely, interorbital breadth does not demonstrate separation among the ancestry groups, with the range of measurement values for Black individuals encompassing that of both the White and Hispanic samples. This suggests, for instance, that relative assessments of nasal aperture to interorbital widths do not strongly inform ancestry estimations, especially since these variables are correlated within individuals.

In summary, these results suggest that certain traits considered in isolation—such as absolute nasal width—can be informative of ancestry. Further, when assessing a particular trait relative to aspects of the facial skeleton, the utility of a given comparative trait varies; certain facial features serve as more appropriate comparative variables for a given trait than others and, thus, should be explicitly included in descriptions of non-metric scoring for applicable traits.

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#### Craniofacial Proportions, Ancestry Assessment, Macromorphoscopic Traits



### A116 Undocumented Border Crossers (UBCs) By Sea: Investigating the Eastern Border Crisis

Amanda N. Friend, MA\*, C.A. Pound Human Identification Laboratory, Gainesville, FL 32608

**Learning Overview:** After attending this presentation, attendees will appreciate the complexity of the Eastern border within the medicolegal context, the difficulty in applying previously successful methodology, and how this relates to the border crisis in the Southwestern United States.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by assessing the challenges posed by the UBCs of the Eastern United States border for forensic anthropologists and the medicolegal field at large.

From 1998 to 2017, the United States Border Patrol (USBP) has reported a total of 6,745 deaths of UBCs in the Southwest sectors.<sup>1</sup> As many who work in these sectors have noted, this is likely a gross underrepresentation.<sup>2,3</sup> Even less represented are decedents from the Eastern border as deaths are not tracked or reported from the USBP for the Coastal sectors including the Miami Sector. The Miami Sector is the focus of this presentation as it is demonstrably the largest point of undocumented immigration attempts.<sup>4</sup> This has resulted in a large knowledge gap in the understanding of the complete human rights implications occurring in the United States border regions.

Unlike the Arizona or Texas border sectors, there are no known isolated regions where deceased UBCs are found or buried in the Miami Sector. As a result, there is no clear population with which to make comparisons between the Southwest and Coastal border sectors. This lack of geographic separation between UBCs and other forensic populations potentially causes difficulties in even identifying an unknown decedent as a UBC prior to identification. Compounding this is the large number of migrants that were granted legal status in the United States due to previous immigration exceptions, such as the Haitian refugee status or the “wet foot, dry foot” policy for Cubans that are likely to also comprise a large portion of Miami Sector UBCs.<sup>4</sup> This then creates a lack of morphological distance between the local forensic and the UBC populations.

One successful method for identifying potential UBCs in a Medical Examiner Office (MEO) setting has been developed in the Southwest by Anderson working at the Pima County MEO in the Tucson Border Sector.<sup>5</sup> This method involved the creation of a UBC profile to screen unidentified decedents that went through the Pima County MEO. To construct an artificial Miami Sector UBC population and qualitatively assess the methodology of screening for UBCs from a forensic population, this study constructed an expected Miami Sector UBC profile. The constructed profile included biological, geographical, and possible expected associated artifacts based on the UBC and Florida migration literature. This profile was then used to screen cases from the four southernmost MEO districts that had been processed at the University of Florida C.A. Pound Human Identification Laboratory (CAPHIL) since the beginning of casework in 1972 to 2018.

The screening resulted in a minimum of 20 cases flagged as potential UBCs. This small sample is likely due to several methodological problems that create difficulty in applying successful approaches in the Southwest to the Miami Sector. Lack of associated documentation in reports on artifacts recovered with the remains or clear documentation of recovery context could have resulted in exclusion of UBCs. Limitations within typical forensic anthropological analyses may have excluded possible UBCs due to lack of informative elements or lack of appropriate reference populations for ancestry analyses (i.e., Caribbean samples), resulting in a failure to meet the criteria set out in the constructed Miami Sector UBC profile. Furthermore, the terrain that UBCs traverse to enter the United States via the Miami Sector is a large body of water, which likely makes recovery highly unlikely. A further potential reason for this screening process returning a limited number of potential UBCs is that potential (or confirmed) UBCs may have been exclusively recovered and evaluated at the MEOs and thus were never sent to the CAPHIL for analysis. Conversely, the required broadness of the ancestry component of the Miami Sector UBC profile may have resulted in non-UBC individuals erroneously flagged as potential UBCs. The results of this analysis demonstrate the complexity of the challenge faced by forensic anthropologists and MEO personnel investigating forensic cases, including UBCs in the Miami Sector, and how these challenges are different than those working on border crisis in the Southwest.

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#### Undocumented Border Crossers, Miami Sector, Identification



### A117 Examining Differences in Presumed Migrants From Texas and Arizona Using Cranial and Dental Data

Christopher A. Maier, PhD\*, Eckerd College, St. Petersburg, FL 33711; Rebecca L. George, MA, University of Nevada, Reno, Reno, NV 89506

**Learning Overview:** After attending this presentation, attendees will understand the variation in cranial morphoscopic and dental morphological traits in samples of presumed migrants from Texas and Arizona, as well as how this relates to potential differences in region of origin between the two samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring the effect of geography on the expression of traits commonly used in the estimation of ancestry in a forensic context. Additionally, it will contribute to the understanding of skeletal variation in the migrant populations increasingly encountered in forensic casework.

Immigration to the United States from Mexico and other Latin American countries has increased over the past several decades.<sup>1,2</sup> As the result of immigration policies enacted in the 1990s, migrants have been forced to more dangerous routes into the country.<sup>3,4</sup> In particular, routes through the Sonoran Desert into Arizona and through south Texas have become increasingly well-traveled.<sup>5-7</sup> The higher volume of migrants on these inhospitable routes has led to an increased appearance of presumed migrant remains in the work of forensic anthropologists.<sup>6,8</sup> These anthropologists are then faced with the task of identifying the deceased.

The system of racial and ethnic classification typically employed in the United States classifies these migrant individuals under the umbrella term “Hispanic.”<sup>8,9</sup> However, recent research suggests that migrants coming through the Arizona corridor are predominantly from northern and western Mexico, while those entering the United States through Texas originate in central and eastern Mexico and other Latin American countries.<sup>5,7,10-12</sup> Several studies have demonstrated craniometric differences between Mexican and “non-Mexican Hispanics”, as well as genetic and craniometric differences between western and eastern Mexico.<sup>7,13-16</sup> It is unknown to what degree these differences affect other features used in ancestry estimation, such as cranial morphoscopic traits and dental morphology.

This project is based on a sample of presumed and known migrant deaths from both the Pima County Office of the Medical Examiner (PCOME) in Arizona and as part of Operation Identification (OpID) in Texas ( $n=238$ ). Although many of these individuals have not been positively identified, their status as presumed migrant deaths is typically ascertained from the context of the recovery and the condition of the remains, as well as skeletal evidence of the biological profile.<sup>5-8,17</sup> Cranial morphoscopic traits and dental morphology were recorded following published standards.<sup>18-22</sup> Individual trait frequencies were examined for significant differences between the two samples using chi-square tests. Additionally, dichotomized dental morphology data were used to evaluate differences between the two samples using the Mean Measure of Divergence (MMD).

Nasal bone contour and zygomaticomaxillary suture shape are the only morphoscopic traits that are significantly different between groups. Individuals from OpID tend toward higher scores, between 3 and 4, for nasal bone contour, while those from the PCOME have more moderate scores for this trait. A score of 0 is more common among OpID individuals for zygomaticomaxillary suture shape, whereas individuals from the PCOME more frequently exhibit a score of 2 for this trait.

Of the individual dental traits examined, only premolar accessory cusps and the protostylid are significantly different between groups. When dichotomized, six dental morphological traits are significantly different between samples and used in the MMD analysis (premolar accessory cusps, hypocone, maxillary enamel extensions, cusp 6, and mandibular molar crenulations). Based on these traits, the MMD indicates that the two groups are significantly different. Several of the significantly different traits (e.g., protostylid, enamel extensions, cusp 6) are more common in Native American populations and are found at higher frequencies in the sample from OpID.<sup>23</sup> This may indicate that individuals in that sample have a greater contribution of Native American ancestry than those from PCOME.

Although the individuals represented by these two samples are jointly classified as “Hispanic” in the United States, there are differences between them in cranial morphoscopic trait frequencies and dental morphology. While these differences are of a smaller magnitude than is observed between other groups, the results support the reconsideration of utilizing an umbrella term for “Hispanics.” Genetic and craniometric data have also found significant differences between similar samples as those used in this study, providing further evidence for applying regional classifiers to “Hispanic” populations.

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### Forensic Anthropology, Ancestry Estimation, Hispanic Ancestry



### A118 A Mixed-Method Approach to Predicting Deceased Migrant Attributes in the Southern Arizona Desert

Caitlin C.M. Vogelsberg, MS\*, Michigan State University, East Lansing, MI 48824

**Learning Overview:** After attending this presentation, attendees will better understand methods developed in several disciplines as they are applied to the predictive modeling of attributes of Unidentified Border Crossers (UBCs) recovered in the southern Arizona desert.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the utility of employing traditional methods of ancestry prediction using skeletal traits in a geospatial framework to aid in the identification of UBCs. These methods could be applied to similar situations in which the identification process may be supplemented by an individual's physical location on the landscape.

The ongoing humanitarian crisis along the southern United States border demands the expansion of methods used to identify unknown individuals found in this region. The correlation between the ancestral background of an individual and their skeletal morphology has been well established and now the implications of combining these data with other indicators of geographic origin must be investigated. Research into the spatial relationships of identified UBCs recovered in the southern Arizona desert has shown positive spatial autocorrelation between attributes such as a person's sex and country of origin.<sup>1</sup> These findings demonstrate that where an individual is from influences where they are found after dying while crossing the United States-Mexico border. This has been documented in the sociological and ethnographic literature and can now be seen in the forensic anthropological data as well. This research applies these spatial patterns to the identification process to predict the country of origin of an unknown individual.

Geographically Weighted Regression (GWR) analyses using the R package *spgwr* were employed to create a predictive model for an individual's country of origin using skeletal indicators of ancestry and the recovery location of remains.<sup>2</sup> Rather than creating a single equation like traditional regression methods, GWR calculates the model at each data point relative to both its physical location in space and to the other points around it. The global model applied to the GWR analyses used factor analysis of craniometric and macromorphoscopic data from 25 identified individuals originating in Mexico and Guatemala who were previously analyzed at the Pima County Office of the Medical Examiner (PCOME). For each case, accurate Global Positioning System (GPS) coordinates of their recovery location in the southern Arizona desert were known. The GWR model ( $R^2=0.540$ ) was developed using this known dataset and accounted for just over half of the variation in the sample. This is an increase from the global model ( $R^2=0.432$ ), which did not incorporate the recovery location and attributes of other individuals found nearby. The GWR model also had lower residual squares values compared to the global model, which indicates a smaller difference between the observed values and the predicted values calculated using the GWR. Other indicators of model goodness-of-fit, including low Root Mean Squared Prediction Error (RMSPE) and Mean Absolute Prediction Error (MAPE) rates show that more accurate country of origin predictions also were created using the GWR method.

The model was then tested on a set of individuals ( $n=8$ ) with presumptive identifications of Mexican origin. The test correctly predicted the country of origin of two individuals and provided promising results for future predictive modeling. Although sample sizes were small, the potential for successfully applying this method was shown. Furthermore, the application of these techniques to other situations in which the physical location of an individual might correspond with their personal characteristics is demonstrated.

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#### Undocumented Border Crossers, Geospatial, Migration





### A119 A Validation of the 2014 Hefner and Ousley Decision Tree Model for Ancestry in a Mexican Population

Bersal C. Villegas-Camposeco, *Escuela Nacional de Antropología e Historia, Mexico City 14030, MEXICO*; Mackenzie Walls\*, *Forensic Anthropology Program, Topeka, KS 66621*; Antinea Menéndez Garmendia, *MS, Universidad Nacional Autónoma de México, Mexico City 04510, MEXICO*; Gabriela Sánchez-Mejorada, *PhD, Universidad Nacional Autónoma de México, Ciudad de México 04510, MEXICO*; Alexandra R. Klales, *PhD, Washburn University, Topeka, KS 66621*; Jorge A. Gómez-Valdés, *PhD, Instituto Nacional de Antropología e Historia, Ciudad de Mexico 14030, MEXICO*

**Learning Overview:** After attending this presentation, attendees will understand the decision tree method of ancestry estimation performed in a distinct Hispanic sample from outside the United States.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reaffirming the need of greater population-specific samples to develop and validate ancestry estimation methods currently used in forensic casework.

Accurate estimation of ancestry plays an important role for human identification and is the most complex parameter of the biological profile to assess. As the Hispanic population of the United States increases, so must our methods so that we may accurately estimate ancestry of the different populations encompassed as “Hispanic.” This study examines the validity and reliability of the decision tree model of ancestry estimation proposed by Hefner and Ousley to determine if it works in an independent sample of Mexican individuals.<sup>1</sup> The decision tree method addresses issues of standardization and uses trait frequencies of the Anterior Nasal Spine (ANS), interorbital breadth, Inferior Nasal Aperture (INA), nasal aperture width, and nasal bone contour to differentiate between White, Black, and Hispanic individuals.

For this research, these five morphoscopic traits, utilized in the Hefner and Ousley decision tree, were scored and utilized to estimate group membership by the decision tree model in a sample of 75 individuals from the Universidad Nacional Autónoma de México Collection, housed at the Departamento de Anatomía, Facultad de Medicina collected previously in research by Villegas-Camposeco.<sup>1,2</sup> The collection is comprised of contemporary mestizo individuals from Mexico City (1990–2010 date of death) that are unclaimed bodies from forensic, public hospitals, psychiatric institutions, and shelters. A subsample of 20 individuals was scored by the first observer, with six months between scoring events, to test intra-observer error, then by a second observer to test inter-observer error using the Intraclass Correlation Coefficient (ICC) and weighted Kappa (*wK*).

Classification accuracy using the decision tree was low at 18.7%. Within the decision tree, INA must always be a score of 2 (moderate sloped) or 3 (straight), and the ANS must be 3 (pronounced) to classify as Hispanic; however, in this sample, most individuals only showed a slight (score 1) or intermediate (score 2) ANS, and the INA scores varied from a pronounced slope (score 1) up to a pronounced sill (score 5). The variation in the degrees of expression of the features relies on greater frequencies for classification in the decision tree, which would not be considered a misclassification, but rather that it corresponds to the biological diversity of the population. Intra-observer agreement was moderate for all traits except the interorbital breadth (*wK*=.23), with scores typically increasing to a higher (wider) score when scored in the second observation (*n*=8). The inter-observer error was comparable with all agreements being good or excellent, again with the exception of the interorbital breadth (ICC=.27). In 75% of cases, the second observer scored the interorbital breadth as lower (more narrow) than the first observer.

In conclusion, the reliability results indicate general consistency on trait scoring among observers and is consistent with previous research, while the validity results suggest that the decision tree method developed by Hefner and Ousley using a Hispanic sample from the PIMA County Medical Examiner’s Office is not very accurate when applied to this Mexican sample and fails to encompass the range of variation in trait frequencies in this sample.<sup>1</sup> Despite the low classification accuracy with this sample, the trait frequencies are highly concordant with those reported in Hefner.<sup>3</sup> These findings also corroborate previous findings by Hefner and colleagues concerning the “unique population histories” of Hispanic populations.<sup>4</sup> Refinement of the decision nodes and a larger sample of Hispanic individuals may increase accuracy of the decision tree model in Mexican populations.

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#### Mexican Population, Ancestry Estimation, Hefner Decision Tree



### A120 Spatial Distributions of Stable Isotope Ratios in Tap Water From Mexico for Region of Origin Predictions of Unidentified Border Crossers

*Saskia Ammer, MSc\*, Munich, Bavaria 80999, GERMANY*

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**Learning Overview:** The goal of this presentation is to determine the accuracy in a reference database of isotope ratios of tap water from Mexico for predicting region of origin of deceased undocumented border crossers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting novel approaches and data on regionally different isotopic values from Mexican tap water as a tool to predict region of origin for unidentified foreign nationals who died along the United States-Mexico border.

As part of this larger project, 306 tap water samples from 51 towns and cities were collected throughout Mexico during a six-week period. At least three samples were collected from each locale to evaluate local variation as well as interregional variation within Mexico. Ninety-five hair samples from 30 towns were collected as well. Furthermore, 55 samples from 16 unidentified border crossers currently held at the Pima Country Office of the Medical Examiner in Tucson, AZ, were collected. Samples were analyzed at the Vrije Universiteit Amsterdam (Netherlands) and the Stable Isotope Ratio Facility for Environmental Research/Spatio-Temporal Isotope Analytics Lab (SIRFER/SPATIAL) at the University of Utah using Thermal Ionization Mass Spectrometer (TIMS) and Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS).

In this presentation, the results of the oxygen and hydrogen isotopic data will be presented. To display the isotopic variation of Mexico, the results were used to create multi-layered isoscapes for the two isotopic elements in the tap water samples using arcGIS®. Furthermore, the isoscapes using oxygen and hydrogen generated from proxy data were compared to the theoretical isoscapes derived from precipitation records and geological maps. This comparison to existing data was used to explore the potential improvement of models that have been developed previously.

The goal is to incorporate the data into a larger network of tap water and human hair isoscapes to establish probability densities for exploring the most probable regions of origins for the deceased undocumented migrants. Therefore, the isotopic results from ten samples of known origin were overlaid with the established isoscapes to test the model and evaluate the accuracy of the predications in the derived isoscapes. The samples consist of ten teeth, five of which are archaeological specimens and five from identified forensic anthropological cases.

Furthermore, this presentation will discuss the challenges in tap water collection in Mexico as well as the use of tap water for the development of isoscapes. This presentation sets the stage for the remaining parts of this research, the development of human hair and tap water isoscapes for a variety of isotopic elements to aid the identification of deceased undocumented migrants. This will be accomplished by presenting a multi-isotope approach that provides sufficient points of reference to narrow the geographic origin of the deceased undocumented migrants. Overall, it is expected that the diligent multidisciplinary approach of forensic anthropology, biochemistry, hydrology, and geostatistics will together provide all the necessary information to ultimately repatriate the remains of the deceased.

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#### Isotope Analysis, Undocumented Border Crossers, Forensic Anthropology



## A121 Using Semimechanistic Modeling for the Prediction of Oxygen and Hydrogen Drinking Water Isotopes From Human Hair in Mexican Populations

Chelsey A. Juarez, PhD\*, Department of Anthropology CA State Fresno, Fresno, CA 93740

**Learning Overview:** The goals of this study are to: (1) explore the relationship between oxygen ( $\delta^{18}\text{O}$ ) and hydrogen ( $\delta^2\text{H}$ ) in tap water samples and human hair samples of known Mexican origin; and (2) explore the utility of semimechanistic modeling of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  of drinking water from hair samples of Mexican origin.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting data on the relationship between Mexican tap waters and human hair and the utility of this relationship to act as a predictor of region of origin.

The use of oxygen and hydrogen isotopes in human drinking water, hair, bone, fingernails, and teeth has demonstrated the ability to successfully track movement and identify the region of origin for modern populations in the United States, Europe, and Asia.<sup>1,2</sup> Bowen et al. published a mass balanced semimechanistic model with adjustable parameters to estimate the value of  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in drinking water based on  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in human hair. This study tests the ability of this model to predict region of origin by estimating the oxygen and hydrogen isotopes associated with drinking waters for Modern Mexican populations

A subset of samples was used for this study, including  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  for  $N=38$  hair samples and  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  for  $N=80$  water samples from six states and overlapping locations in Central and Southern Mexico. Hydrogen and oxygen isotopes were measured for all samples for all locations using laser absorption spectroscopy at the University of Utah Stable Isotope Ratio Facility for Environmental Research (SIRFER) laboratory. The results are reported here using delta notation and the Vienna Standard Mean Ocean Water (VSMOW) scale. MATLAB<sup>®</sup> 9.2 was used to generate a semimechanistic mass balanced model based on the work of Bowen et al. (2009) using known  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  from hair values. General statistics were analyzed using SPSS version 25.0.

**Results:** Tap water values spanned a range from +0.4‰ to -12.7‰ and -4.2‰ to -91.7‰ for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$ , respectively. Oxygen values for human hair ranged from 15.5‰ to 9.5‰. Hydrogen values for human hair ranged from -54.4‰ to -90.8‰. Linear regressions of hair and water samples revealed medium to weak correlations, which were lower than those previously published for other regions ( $\delta^2\text{H}_h = -51.678 + 0.347 * \delta^2\text{H}_w$ ,  $R^2=0.65$ ;  $\delta^{18}\text{O}_h = 16.256 + 0.342 * \delta^{18}\text{O}_w$ ,  $R^2=0.32$ ).<sup>2</sup> For the model, the following parameters were adjusted to maximize fit,  $F_s=0.18601$ ;  $l=0.00022849$ ;  $g_1=0.64281$ ;  $ahw=1.196$ ;  $aow=1.0125$ . Despite these adjustments, the medium and weak correlations between hair and estimated drinking water values remain.

The inconsistent results between this study and those on other regions suggests shifting water usage over time and the presence of multiple inputs in the diet of Mexican populations across states. Mexico suffers from water stress and, as a result, has the highest consumption of bottled water per capita in the world. This study clearly demonstrates that in order to use water isotopes in Mexican hair to determine region of origin, these cultural contexts must be taken into consideration.

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### Geolocation, Isotopes, Hair



### A122 Predicting Region of Origin for Unidentified Deceased Migrants at the Texas-Mexico Border Utilizing Stature and Stable Isotopes

*Nicholas P. Herrmann, PhD\**, Texas State University, San Marcos, TX 78666; *Robyn Theresa Kramer, MA*, Chico, CA 95928; *Christopher A. Wolfe, MA*, University of Nevada, Reno, Reno, NV 89557; *Kate Spradley, PhD*, Texas State University, San Marcos, TX 78666

**Learning Overview:** The goal of this presentation is to predict the region of origin for unidentified deceased migrants at the United States-Mexico border using stature data obtained from country-specific national health and nutrition surveys across Mexico and Central America, stable isotope models for strontium and oxygen of the same region, and a maximum likelihood assignment model that will utilize spatial statistics to predict an estimated region of origin.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting interdisciplinary approaches to both forensic identification and their application to human rights issues at the border and beyond by integrating traditional forensic and biological anthropological methods, such as stature estimation, with biogeochemistry and geostatistics to improve the probability of identifications of these challenging cases.

Stature varies markedly across Mexico and Central America and can be combined with previous studies of isotopic variation to narrow region of origin of unidentified migrants found at the United States-Mexico border. Establishing region of origin can reduce the potential matches for unknown cases within missing persons databases and potentially aid in generating positive identifications for deceased migrants recovered along the United States-Mexico border.

**Hypothesis:** A dual-isotope isoscape and likelihood assignment method can be used in conjunction with known stature data to estimate region of origin for deceased migrants recovered along the Texas-Mexico border and improve the probability of making a positive identification.

**Outcomes:** This presentation will shed light on the humanitarian crisis occurring at the United States-Mexico border and promote interdisciplinary approaches to both forensic identification and its application to human rights issues at the border and beyond by integrating traditional forensic and biological anthropological methods, such as stature estimation, with biogeochemistry and geostatistics, thus improving the probability of identifications of these challenging cases. An additional outcome is closure for the families whose loved ones are still missing along the United States-Mexico border.

**Synopsis/Methods:** Stature data is obtained from country-specific national health and nutrition surveys from across Mexico and Central America. These include Mexico ( $n=22,231$ ), Nicaragua ( $n=15,266$ ), Honduras ( $n=22,757$ ), Guatemala ( $n=25,914$ ), and El Salvador ( $n=7,132$ ). These data are used as an additional predictor variable for a recently tested assignment model that used strontium and oxygen isoscapes for Mexico, Central America, and the Caribbean to predict region of origin for unidentified deceased migrants from the United States-Mexico border. Using the Operation Identification (OpID) forensic cases at Texas State University, previously sampled migrants ( $n=3$ , two identified and one unidentified) are run through the adjusted assignment model to determine if stature is a suitable variable that can narrow region of origin predictions for the OpID cases.

**Results/Conclusions:** The goal of the research is to predict regions of origin for each case and determine whether adding the stature variable aided the prediction. Two of the three samples have been identified since the initial study and the accuracy of the adjusted model predictions can be assessed. Overall, the assignment approach proves successful in narrowing the region of geographic residence for deceased migrants recovered near the United States-Mexico border. Adding stature data to the model improves the method by further narrowing predicted regions of origin and increasing the chance of making a positive identification.

#### Geographic Origin, Stable Isotopes, Stature



### A123 Early Bone Healing Response in an Acute Occipital Skull Fracture of a 19-Month-Old After a 72- to 78-Hour Survival Interval

Dana Austin, PhD\*, Tarrant County MEO, Fort Worth, TX 76104-4919; Maryvi Gonzalez-Sola, PhD, West Coast University - Dept of General Education, Dallas, TX 75247; Susan J. Roe, MD, Tarrant County MEO, Fort Worth, TX 76104

**Learning Overview:** The goal of this presentation is to demonstrate through case example the timing of fracture repair in a pediatric occipital fracture. Subperiosteal new bone is observed macroscopically in this case in which the death occurred after an interval ranging from 72 to 78 hours following cranial fracture in a 19-month-old.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a time frame for early bone response in a pediatric skull fracture using casework with a documented timeline.

This case study follows a 19-month-old female with an acute occipital fracture. The patient was medically treated for 42 hours prior to pronounced brain death and survived 30 additional hours on a ventilator awaiting organ harvesting. The perpetrator had sole custody of the child for six hours prior to the 911 call. Autopsy findings included an acute occipital skull fracture terminating in the foramen magnum, subgaleal, periosteal, subdural, and subarachnoid hemorrhages, cerebral edema with uncal and cerebellar herniation, bilateral multi-layered ocular hemorrhages, scattered contusions, and healing fractures of the right radius and ulna. An anthropology examination using a stereomicroscope revealed Subperiosteal New Bone Formation (SPNBF) in a diffuse pattern surrounding the cranial fracture site and no evidence of bony remodeling of the fracture gap. Other anthropology findings included a healing fracture of the right ulna with separated cortical walls, bridging callous and subperiosteal new bone formation surrounding the fracture, and a healing right radius fracture presenting as a swelling of the cortex with the cortical walls intact.

Fracture healing rates in human infant/child crania have few references in the literature, although it is reported that infants and children have a faster healing rate with deposition of new bone occurring within hours of injury.<sup>1,2</sup> The cranium undergoes intramembranous bone repair, a process that requires no cartilaginous precursor. Early events include cell proliferation and differentiation. Osteoprogenitor cells, including undifferentiated stem cells that convert to osteoblasts, are present in the cambium layer of the periosteum. New bone matrix is synthesized by osteoblasts under the periosteum and deposited onto the cortical surface adjacent to the fracture and progresses toward the fracture.<sup>3,4</sup> The immature dura mater has been shown to produce higher rates of osteogenic cellular activity that contributes to the rapid repair of cranial trauma.<sup>1</sup> It is suggested that the interaction of vascular components and the initiation of the periosteal response facilitate intramembranous bone formation.<sup>4</sup> Histologically, intramembranous ossification is shown to occur a few days following injury.<sup>5</sup> Hard callous is found to develop under the periosteum as early as two to five days in rats.<sup>6</sup>

The knowledge of the timing of the healing response is primarily based on published radiographic data that highlights the time of appearance of SPNBF.<sup>7</sup> It is hypothesized that macroscopic observation will reveal bony change earlier in the process. Anthropologists and pathologists working in the medical examiner setting have the diligence, dedication, and devotion to collect gross and histologic data from cases with known survival intervals to ascertain the timeframe for early healing response in subadult cranial fractures. When interpreting findings of fracture healing in a forensic report, practitioners can report only to the limits of the knowledge shared through publication. This limits the narrow time frame provided to investigators and the judicial system. This presentation provides a documented time frame for the formation of SPNB in a healing cranial fracture and begins a study of known forensic cases to more accurately define time-since-injury estimates.

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#### Subperiosteal Bone Formation, Fracture Healing, Pediatric Skull Fracture



### A124 Fracture Surface Characteristics for the Interpretation of Peri-Mortem Blunt Force Fractures in Bone

Christopher W. Rainwater, MS\*, OCME, New York, NY 10016; Derek Congram, PhD, International Committee of the Red Cross, Bogota, D.C., COLOMBIA; Steven A. Symes, PhD, MS Medical Examiner's Office, MCL, Pearl, MS 39208; Nicholas V. Passalacqua, PhD, Western Carolina University, Cullowhee, NC 28723

**Learning Overview:** The goal of this presentation is to define and explain two peri-mortem blunt fracture characteristics in bone and to demonstrate their utility in assessing direction of failure using biomechanical principles, particularly highlighted in remains that have been significantly altered by taphonomic processes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing explanations of blunt fracture characteristics that assist in the interpretation of force and fracture directionality, which can aid in the determination of cause and manner of death. The cases presented will also help clarify distinctions between peri-mortem trauma and postmortem damage.

The interpretation of peri-mortem traumatic injuries to the skeleton may be one of the most difficult and important aspects of forensic anthropological analyses. In cases in which the remains have been skeletonized, the hard tissues are the only record from which cause and/or manner of death can be elucidated. In order to properly analyze skeletal trauma, one must understand not only bone biomechanics, but also take into consideration the shape and size of the impacting implement, as well as the impacting force. Further, in order to fully characterize the bony defects, a thorough taphonomic analysis in which peri-mortem fractures are distinguished from postmortem (taphonomic) damage must also be completed.

Once skeletal elements have passed from the peri-mortem interval and lost their viscoelastic properties, postmortem fractures differ from their peri-mortem counterparts, even under similar loading conditions. Instead of straining to slow the applied loading forces, “dry” bone is relatively brittle and unable to resist the load. As the bone failure progresses through tension, shear, and compression in less time than fresh bone, the resulting postmortem fracture features tend to be rough, jagged, and lack the large peak and valley notches characteristic of fresh bone failure.

The goal of this presentation is to define, explain, and test the application of two peri-mortem blunt fracture characteristics in bone to contribute to the determination of peri- or postmortem timing of the fracture: *bone tear*, and *bone spurs and notches*. To interpret these characteristics, an understanding of bone biomechanics, primarily the forces of tension and compression is required. In general, forensic anthropology terms, tension forces can be understood as pulling bone apart, while compression forces push bone together. Initial failure in dense cortical bone is usually indicative of tension and the traditional interpretation of blunt force butterfly fractures exemplifies using the utility of tension and compression forces to assess directionality. Here, it is argued that fracture surface morphology adds additional information that can be used to examine peri-mortem blunt fracture circumstances, even in incomplete or poorly preserved remains. Further, it should be noted that other fracture configurations, such as spiral fractures and axial loading, may similarly be interpreted using these characteristics; however, in these cases, the fracture surface morphologies may be more complex.

Two peri-mortem fracture characteristics, *bone tear*, and *bone spurs and notches*, were introduced in Symes et al.<sup>1</sup> However, here we are defining and explaining these features in order to make them standardized and thus increase their diagnostic power in future analyses. *Bone tear* is found on the fracture surface, representing tension forces. This fracture surface will appear smooth, mottled, and wavy, representing the bone being torn apart. *Spurs/notches* are found on the fracture surface representing compression. This fracture surface will rise and fall into sharp peaks and valleys, as this area represents the bone being pushed together until it fails.

These fracture surface characteristics were applied to two cases with depositional periods of approximately 70 years. Results found that in both cases, these characteristics were present and observable, allowing for additional interpretation of the skeletal trauma present on the remains, despite the co-occurrence of significant postmortem damage and weathering. However, additional research is needed, as demonstrated in Christensen et al., and the approach to the analysis of skeletal trauma using fracture surface morphologies is promising.<sup>2</sup>

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#### Blunt Force Trauma, Skeletal Trauma Analysis, Fracture Surface Morphology



### A125 Buckle Rib Fractures: More Than a Pleural Surface Phenomenon

Timothy P. Gocha, PhD\*, Clark County Office of Coroner/Medical Examiner, Las Vegas, NV 89106; Mariah E. Moe, BS\*, Las Vegas, NV 89119

**Learning Overview:** After attending this presentation, attendees will understand the need for using more precise anatomical terminology when documenting buckle rib fractures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a lack of consistency and precision in the definition of buckle rib fractures in the forensic science literature over the past 15 years, in terms of the underlying mechanism of fracture and/or anatomical location of fracture. Suggestions for improving the fracture terminology are made, which should help eliminate confusion and improve the quality of forensic anthropological practice.

In 2004, Love and Symes, citing engineering sources, introduced buckle rib fractures into the anthropological literature, defining them as, “a fracture wherein the bone failed at the point of compressive stress prior to failure at the point of tensile stress.”<sup>1</sup> Despite this straightforward and accurate definition, in the 15 years since its introduction, slight variations of this definition have been presented, leading to possible confusion as to what actually constitutes a buckle rib fracture. To explore these differences, a thorough literature review was conducted. All peer-reviewed sources in English that cited the Love and Symes article were investigated as to whether they referenced buckle rib fractures, whether they defined buckle rib fractures, and if so, how buckle rib fractures were defined.<sup>1</sup>

Overall, 36 articles and book chapters fit the criteria for inclusion in this survey. Of these, 18 (50.0%) referenced Love and Symes for reasons other than buckle rib fractures, while 10 (27.8%) of them specifically mention buckle rib fractures, yet do not define them.<sup>1</sup> Only 8 of the 36 sources (22.2%) mention buckle rib fractures while also providing a definition. Of those eight sources, only five define buckle rib fractures as being the result of failure in compression prior to failure in tension; two sources fail to specify a strain mode responsible for the failure; and one source mistakenly reports that buckle rib fractures are the result of failure in tension. In addition to the loading mechanism responsible for failure, three of the eight sources that define buckle rib fractures also include mention of the rib surfaces involved. Two of the three mention that buckle rib fractures can occur on either the “inner” or “outer” cortex of the rib, while one source defines buckle rib fractures as specifically occurring on the pleural surface of the rib.

A case study will be presented that demonstrates multiple incomplete fractures on the right ribs where the cutaneous surface near the sternal end presents with a sharp disruption in the normal alignment of the cortical bone surface, having a crumpled or kinked appearance, while the pleural surface remains intact. Other trauma throughout the skeleton indicates a significant traumatic impact to the left side of the body. It is hypothesized that the load applied to the left side of the thorax transferred through the left costal cartilages to the sternum and continued through the right costal cartilages such that the sternal end of the right ribs was loaded in bending with the pleural surface in tension and the cutaneous surface in compression. As these fractures seemingly resulted from compressive forces, rather than tensile ones, they are classified as cutaneous buckle rib fractures.

In keeping with the engineering literature from which the term was taken, most authors who define buckle rib fracture define it by the loading mechanism responsible and agree that they are the result of failure in compression prior to failure in tension. However, the majority of sources that mention buckle rib fractures fail to specify the affected rib surface. As the case study presented here demonstrates, buckle rib fractures are not only a pleural surface phenomenon and should not be assumed as such. Under certain loading scenarios, the cutaneous surface of the ribs can be loaded in compression with the pleural surface in tension. Ergo, it is recommended that practitioners specify which anatomical surface is affected, notated as a pleural buckle rib fracture or cutaneous buckle rib fracture, to increase the precision of fracture description.

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**Buckle Rib Fracture, Trauma Analysis, Fracture Analysis**



### A126 Is Puppe's Rule That Flawless? Two Peculiar Cases of Cranial Fractures Running Through Craniotomy Holes

*Alberto Amadasi, MD\**, Università di Bologna, Bologna 40126, ITALY; *Francesca Magli, MA*, LABANOF, Milan, ITALY; *Debora Mazzei, BS*, LABANOF - Sezione di Medicina Legale, Milan 20133, ITALY; *Annalisa Cappella, PhD*, LABANOF - Sezione di Medicina Legale, Milano 20133, ITALY; *Enrico A. Muccino, MD*, LABANOF, Milan 20133, ITALY; *Katiuscia Bisogni, MD*, Vibo Valentia 89900, ITALY; *Cristina Cattaneo, PhD*, LABANOF -Sezione di Medicina Legale, Milan 20133, ITALY

**Learning Overview:** After attending this presentation, attendees will be aware of peculiar exceptions to the Puppe's rule about cranial fractures in two cases where fractures exhibited an unexpected path.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing that the Puppe's rule, which has never been refuted, may have a possible exception, which must be considered when skull fractures encounter previous craniotomy holes.

More than 100 years ago, the German forensic pathologist Puppe established a rule concerning the sequence in which multiple blunt force and injuries of the skull occurred. According to Puppe's rule, when dealing with intersecting fractures, it is possible to devise which injury was first produced as the undamaged skull allows fractures to develop normally while the fractures caused by the subsequent injury are stopped where other fracture lines are already present. This means that fractures from subsequent impacts are arrested at pre-existing fracture lines of the skull. Moreover, Puppe's rule was reliably applied even in cases of gunshot wounds to identify entries and exits. It was even demonstrated that gunshot holes and beveling were interrupted and delimited by pre-existing fracture lines. Moreover, fractures from exit gunshot wounds stop at the edge of the entry wound.

But what happens to a fractured skull when pre-existing holes are present? If bone around the hole's edge is largely remodeled, will this stop the fracture or will something different happen?

Two unusual cases are presented of subjects undergoing cranial fractures due to blunt force trauma (case 1) and gunshot (case 2): both previously underwent neurosurgical operations with the persistence of the holes produced by the craniotomy drill, with remodeled smooth bone around the edge.

Case 1 concerned a single gunshot to the skull of a male with a previous craniotomy hole in the right parietal region, with a large bone remodeling, dating back 15 years. Case 2 concerned blunt force fractures (a fall from standing) in a subject with several widely remodeled craniotomic holes, dating back 20 years earlier.

What was arguable, according to the information provided by the Puppe's rule, was that the fracture lines stopped at the edge of the craniotomy hole. However, what has been detected was different than what was expected. As a matter of fact, fracture lines continued exactly in the opposite direction, as though they were "skipping" the hole, following the same direction and the same axis and stopping a few centimeters over on the opposite side of the craniotomic hole.

This represents a very interesting "exception" to the rule of Puppe, probably determined by different forces of tension in the bone tissues due to bone remodeling, but which must be pointed out and is worth considering in similar cases. It is therefore arguable that remodeled bone behaves completely differently than fracture lines or coeval bone holes.

The Puppe's rule has never been refuted, but these cases are the closest to an exception ever seen in forensic anthropology.

#### Cranial Fractures, Puppe's Rule, Craniotomy Holes





## A127 Using High Resolution Mass Spectrometry Analysis to Investigate Trabecular Bone Metabolomics for Postmortem Interval (PMI) Estimation

Beatrix Dudzik, PhD\*, Lincoln Memorial University, Harrogate, TN 37752; Natalie R. Langley, PhD, Mayo Clinic School of Medicine, Scottsdale, AZ 85259; Kathleen Hauther, University of Tennessee, Knoxville, TN 37996; Lee Meadows Jantz, PhD, University of Tennessee, Knoxville, TN 37996-1525; Taylor Beckmann, BS, Lincoln Memorial University, Harrogate, TN 37724; Johnny E. Cebak, PhD, Lincoln Memorial University, Harrogate, TN 37752; Michelle Donohue, BS, Lincoln Memorial University, Harrogate, TN 37724; Corey Hoch, BS, Lincoln Memorial University, Harrogate, TN 37724; Stacy Chelf, MS, Lincoln Memorial University, Harrogate, TN 37724; Paul Wood, PhD, Lincoln Memorial University, Harrogate, TN 37752

**Learning Overview:** After attending this presentation, attendees will better understand the potential of quantifying lipid breakdown in trabecular bone for PMI for long periods of time following soft tissue decomposition (years to decades).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the preliminary results of lipidomic analysis of bone biopsy samples from 115 individuals with postmortem intervals that range from less than 1 year to 30 years.

This study expands on previous research that established quantitative approaches for examining skeletal muscle and trabecular bone metabolites using high resolution mass spectrometry to examine long-term preservation lipid candidates in bone. As lipids have shown to be preserved in bone marrow for long-term intervals, from several months to several decades postmortem, further quantification of lipid degradation in bone marrow will aid in building and validating regression equations using metabolite ratios to estimate the postmortem interval of skeletonized remains, which is notoriously difficult for forensic practitioners.<sup>1-3</sup>

Bone biopsy samples of fresh and skeletal donors with varying postmortem intervals (<1 year–30 years) were subjected to high resolution mass spectrometry to identify preserved lipid biomolecules in bone, which, upon further analysis, will be used to test their capacity to accurately predict long-term postmortem intervals (e.g., years or decades) from skeletal remains.

Twenty fresh human donors were placed at the University of Tennessee Anthropological Research Facility in two cohorts. Ten fresh donors were placed in late January 2018, and an additional ten fresh donors were placed in mid July 2018. Ninety bone biopsy samples have so far been obtained from this experimental cohort to track any significant changes in lipid content that may be correlated with soft tissue decomposition. These donors will continue to decompose, and bone biopsy samples will be collected every six months through the end of 2019. Bone biopsy samples were additionally taken from a cross-sectional sample represented by dry skeletal material of 115 individuals curated at the William M. Bass Donated Skeletal Collection, with postmortem interval ranges of 1 to 30 years.

A total of 435 bone biopsies were taken from 135 individuals from three skeletal sites with high trabecular bone content (calcaneus, proximal tibia, and vertebral body) from the combination of experimental and cross-sectional samples (fresh and skeletal, respectively). Of the 435 bone biopsy samples taken from 135 individuals (this *n* refers to the fresh and skeletal donors), bone biopsy material from 99 donors have undergone an organic extraction process and have been subjected to mass spectrometry analysis. Relative intensities (ratio of lipid classes identified in samples compared to internal standards) have been recorded and banked in a Microsoft® Access® database.

In a previously reported pilot study, the diversity of a class of lipid mediators and bone metabolism regulators, N-acyl amino acids, were investigated in a dry human calcaneus with a PMI of approximately seven years.<sup>4</sup> Utilizing a high-resolution electrospray ionization lipidomics analytical platform, 76 potential N-acyl amino acids were identified in the seven-year PMI sample, providing the impetus for a large-scale study. The structural identities of palmitoyl and oleoyl serine were validated via generation of the MS<sup>2</sup> product ion for serine (<1ppm mass error), and 20 additional lipid class candidates are currently being validated using Tandem Mass Spectrometry (MS/MS) using the 435 bone biopsy samples from 115 skeletal donors. Upon validation, the ratio of lipid intensity compared to internal lipid standards allows for standardization and interpretation of degradation of lipids and associated metabolites over time.

This study expands the previous research on skeletal muscle metabolites to bone<sup>5</sup>, and provides a destructive, yet minimally invasive, bone biopsy sampling technique that will ultimately be used to build a validated method for estimating PMI of skeletonized remains based on lipid degradation in bone over significant time periods following soft tissue breakdown.<sup>5</sup>

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### Postmortem Interval, Metabolomics, Mass Spectrometry



## A128 Assessing the Accuracy of Decomposition Scoring Methods in Arizona's Sonoran Desert

Shari G. Ex, BA\*, University of Tennessee Knoxville, Knoxville, TN 37996; Jason P. De León, PhD, University of Michigan - Dept of Anthropology, Ann Arbor, MI 48109

**Learning Overview:** After attending this presentation, attendees will have a unique insight into which decomposition scoring methods for estimating Postmortem Interval (PMI) are most effective within the geographic region of the southern Arizona desert at the United States-Mexico border.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating why the development of observationally based scoring methods within a statistically grounded model is necessary in forensic contexts.

The primary purpose of this research was to investigate whether existing methods of decomposition assessment can accurately estimate PMI in the southern Arizona environment to more effectively address the migrant death crisis.

In the past five years, more than 1,500 migrants have died in the attempt to cross the United States-Mexico border.<sup>1</sup> As forensic anthropologists and medical examiners attempt to identify these individuals by compiling biological profiles and estimating PMI, the use of accurate methodology is crucial. Megyesi et al. has become the standard method of quantitatively assessing decomposition.<sup>2</sup> Their approach calculates a point-based Total Body Score (TBS) that predicts Accumulated Degree Days (ADD) and, thereby, PMI.

A significant drawback of this method is its creation based on photographs of human remains and not from first-hand, systematic observational research of the processes of decomposition *in situ*. TBS is a modification of a similarly retrospectively developed method intended to assess decomposition in southern Arizona.<sup>3</sup> However, TBS assumes strict sequential processes of decomposition that are not necessarily reflected in observational studies and do not account for animal scavenging of remains. The TBS method has also been modified specifically for use on pig models to account for observed differences between species.<sup>4</sup> The Geographic Information Systems (GIS) method attempts to account for variability in decomposition by separating the body into 16 regions and assessing the presence or absence of a comprehensive trait list.<sup>5</sup> Overall, there is a significant dearth of observational studies from southern Arizona's Sonoran Desert. The present investigation explores the accuracy of the three aforementioned approaches (TBS, modified TBS, and GIS) for estimating PMI in this region.

Since the state of Arizona does not have a research facility with a human donor program, pig models (*Sus scrofa*) were used in the current study. Although pigs are not an idyllic proxy for predicting PMI in humans, they serve as acceptable proxies when pursuing specific research endeavors.<sup>6</sup> Such endeavors include comparing the efficacy of scoring methods in specific environments. Additionally, consistently warm temperatures in the summer months result in stronger correlations between pig and human decomposition processes.<sup>6</sup> As such, since the current study was methodologically focused and took place in the summer months of June and July, pigs served as adequate replacements for human subjects.

The experiment took place in Arivaca, AZ, approximately 15 miles from the United States-Mexico border. Four pigs between the weights of 130–170 pounds were euthanized on site and placed approximately ten meters apart in direct sunlight. Two of the pigs were clothed to more realistically portray the circumstances of human death and to test the effects of clothing on decomposition and scavenging activity. Decomposition was scored using the three methods twice per day—once in the morning and again in the late afternoon—until all four pigs were completely skeletonized.

As per Megyesi et al., ADD was calculated by adding the average temperatures of each day in degrees Celsius.<sup>2</sup> To assess the results of the three methods, statistical tests were run using R, and linear regression models were used to estimate PMI based on TBS scores and ADD. Random forest regressions were employed to predict ADD based on data collected through GIS scoring. The data were compared to known PMI to assess the accuracy of each of the three scoring methods. The preliminary results suggest that the commonly employed methods of scoring decomposition vary significantly in their ability to predict time since death along the United States-Mexico border in southern Arizona, with the GIS scoring system showing the most promise.

This research supports the notion that existing methods of TBS-based decomposition assessment do not accurately estimate PMI in the southern Arizona environment. This presentation seeks to emphasize the need for a more robust, context-specific research base when addressing the tragic deaths along the United States-Mexico border.

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### United States-Mexico Border, Decomposition, Migration

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### A129 Deconstructing Non-Carious Cervical Lesions on Teeth in Forensic Contexts

*Jessica I. Cerezo-Román, PhD\**, Department of Anthropology, Norman, OK 73019; *Bruce E. Anderson, PhD, PCOME*, Tucson, AZ 85714

**Learning Overview:** After attending this presentation, attendees will have a better understanding of: (1) variability in non-carious cervical lesions, and (2) the use of non-carious cervical lesions at the cementoenamel junction for the purpose of reconstructing biological profiles, which can help to estimate if the remains are modern or ancient and determine if the remains are of legal significance.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of an integrated approach to analyzing non-carious cervical lesions at the cementoenamel junction to obtain a positive identification and medicolegal vs. archaeological importance of skeletal remains.

The causes of the non-carious cervical lesions at the labial surface of the teeth and its medical-legal implications are examined in detail in this presentation. This study used two forensic anthropological cases to illustrate these lesions and their implications for medical-legal research. The presence of cervical lesions can have long-term health consequences, such as hypersensitivity in the dentine, risk of tooth fracture, the potential for dentine and pulp exposure, inflammation of the area, and tooth loss. The lesions are described as hairline crack striations with a wedge shape and sharply delimited borders, triangular, C-shaped lesions with rounded floors, and saucer-shaped. These types of lesions have medical significance and usually are found and treated by dentists and described in antemortem records. However, the etiologies of the lesions are a fairly contested topic and understudied in forensic anthropological investigation.

Lesions observed in these areas are multifactorial and not caused by a single mechanism. They typically relate to a combination of factors, such as abrasive action from the use of toothpaste combined with over-brushing, diseases, the excessive consumption of acidic food, and the use of toothpicks. Non-carious lesions at the cementoenamel junction are rarely found on archaeological material. However, it is commonly found in forensic anthropological cases.

The methods used to analyze the two case studies focus on reconstructing the biological profiles. Preparation of the elements with soft tissue included several hours of soaking in a heated water bath with equal parts detergent and sodium carbonate ( $\text{Na}_2\text{CO}_3$ ). Any adherent soft tissue was then manually removed from the bones, and all the resected elements were allowed to dry. Photographic documentation of selected remains and skeletal features were taken at different times during the examination. Also, a complete set of DEXIS digital dental periapical radiographs were made during the examinations. The individuals were analyzed using the same methods to obtain comparable results. The biological profile includes estimation of the biological sex and age-at-death and documenting pathological conditions, among other variables.

The biological profile data allowed for reconstructing portions of the social life histories of the individuals for subsequent correlation with particular characteristics and ultimately identification. The non-carious lesions at the cementoenamel junction in the two cases presented contribute to the positive identification of the individuals as they provide an additional line of evidence for the dates of the remains and reconstructing the biological profile. It is argued that the presence of non-carious lesions at the cementoenamel junction can help to estimate if the remains are modern or ancient and determine if the remains are of legal significance. Thus, the presence of non-carious lesions on teeth from human remains is an additional line of information that can aid in the reconstruction of the biological profile and ultimately in the positive identification of individuals.

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#### Teeth, Forensic Anthropology, Non-Carious Lesions



### A130 A Retrospective Study of the Forensic Anthropology Cases Investigated by the Legal Medicine Institute in Rio de Janeiro, Brazil, From 2011 Through 2017

Melina Calmon Silva, MA\*, New Orleans, LA 70119; Marcos Paulo Machado, Instituto Médico Legal Afrânio Peixoto, Rio de Janeiro, BRAZIL

**Learning Overview:** After attending this presentation, attendees will better understand the forensic anthropology practices and the investigated cases at the Legal Medicine Institute in Rio de Janeiro, Brazil.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing new data and case work trends of forensic anthropology in Rio de Janeiro, Brazil, and by providing a resource for comparative research between large metropolitan areas.

The Forensic Anthropology Office (SAFO) was established in the State of Rio de Janeiro in October 2010 within the Afrânio Peixoto Legal Medicine Institute (IML-AP). SAFO IML-AP is connected to the Technical-Scientific Police Department, a branch of the Civil Police of Rio de Janeiro State. Like most Brazilian states, the forensic examination provided by SAFO IML-AP is subordinate to the Public Security Secretary, as it is considered a police unit.

A detailed review of anthropology examination reports performed by SAFO IML-AP between the years 2011 and 2017 was conducted to present a demographic of the cases. A total of 196 cases were reviewed. Only cases that were forensically significant and of human origin were included and considered in the final dataset, totaling 181 cases. The data analyzed from each report included the completeness of the remains received; biological profile; trauma analysis; the site where the individual was found; elements that might contribute to identification; and the method used for identification.

The results reveal that 13.5% of all cases analyzed were comprised of White, or Caucasian. males between the ages of 31 and 50 years old (17/126), thus being the most significant demographic represented.

A total of 154 cases were found to have information regarding the site where remains were found. In 8% of the cases (13/154), the remains were found in Realengo, 7% (11/154) were found in Santa Cruz, and 5% (8/154) were found in downtown Rio de Janeiro City. Therefore, 20.8% (32/154) of the cases that had any information regarding where the remains were found is associated with three specific regions of Rio de Janeiro.

Of the cases examined by SAFO IML-AP, 81.2% (147/181) presented some peri-mortem trauma. The most common type of trauma was blunt force trauma with 23.1% (34/147). However, when correlating type of trauma and location of the injury, complete burned remains composed 14.3% (21/147) of the cases. Projectile (gunshot), and blunt force trauma to the head were the second and third most common types of *trauma x location* examined, being present in 12.9% (19/147) and 12.2% (18/147) of the cases, respectively. It is known that gangs in the favelas of Rio de Janeiro burn individuals in an attempt to hinder identification, which can explain the reason why many cases arrive with trauma caused by fire.

The positive identification of individuals was achieved in 13.8% (25/181) of the cases. In 48% (12/25) of the cases identified, the description for the type of identification method used was odontology. Odontology has been heavily used in the realms of forensic anthropology in Brazil where the professionals who hold the title forensic anthropologist are usually dentists with a specialization in forensic dentistry. The odontology methods used for identification include superposition of antemortem and postmortem images of the individual's teeth and dental X-ray comparison. Individuals who are identified by these methods are usually those who achieved the status of a presumptive identification. The universal health care in Brazil provides odontology services for its citizens, and their dental history can then be accessed by the legal medicine offices. Therefore, when individuals with presumptive identification are examined, their records are checked for dental X-ray comparison. DNA was the method used to identify 32% (8/25) of the cases. In comparison to odontology, DNA analysis is also used when individuals reach a presumptive identification.

It is important to note that Brazil does not have a missing persons nor an unidentified remains databank; additionally, the country also does not have a DNA databank. Consequently, the bureaucracy involved in identifying individuals may cause a delay in the process or even prevent it from happening. The data presented by this presentation provides an overview of the work being performed in Rio de Janeiro and offers an opportunity to discuss future developments of the field in Brazil.

**Legal Medicine Institute, Data, Rio de Janeiro**



### A131 Beyond the Biological Profile: Demographic Patterns in Forensic Anthropology Casework in Honolulu, Hawaii

*Jennifer F. Byrnes, PhD\**, University of Hawai'i - West O'ahu, Kapolei, HI 96707; *William R. Belcher, PhD*, University of Hawai'i - West O'ahu, Kapolei, HI 96707; *Christina G. Mello, PhD*, University of Hawai'i, West O'ahu, Kapolei, HI 96707

**Learning Overview:** After attending this presentation, attendees will better understand demographic patterns of forensic anthropological casework in Hawaii.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how public health issues can affect patterns in forensic anthropology casework and, conversely, how forensic anthropologists can provide information germane to public administration officials.

Few published studies have previously examined regional demographic patterns within forensic anthropology casework. Hypothetically, these types of analyses may reveal insights into how forensic anthropology casework patterns are reflective of regional conditions. The forensic anthropology case files for the City and County of Honolulu provide an informative illustration of this phenomenon. In 2015, the Governor of the State of Hawaii declared homelessness a public health emergency associated with the lack of affordable housing. In accordance with nationwide epidemiological statistics, people who experience homelessness tend to have a variety of health issues, ranging from physical to mental. This presentation examines 15 years of forensic anthropology casework from the City and County of Honolulu, Department of the Medical Examiner, to illustrate the use of forensic case files as a source of information on the embodied osseous consequences of the homelessness crisis. Importantly, these data have the potential to provide demographic and other information regarding marginalized or otherwise forgotten members of society.

For the purposes of this study, the Honolulu Medical Examiner's Office granted access to their database of all the cases ( $n=27$ ) that involved a forensic anthropology consultation from 2004 to 2018. The cases were coded for elements of the biological profile, specifically sex, age, ancestry, and antemortem trauma. They were further coded based on the medical examiner's conclusions of manner of death. Lastly, if personal identity was determined, then additional information was coded for the decedent's residency and/or homelessness status, mental illness(es), and drug abuse/use. Documented chronological age at death superseded biological age estimates.

The results suggest that known public health issues likely affect patterns in forensic anthropology casework in the City and County of Honolulu. The highest frequencies by category were males (70%), middle-aged from 35 to 55 years (46%), and of European or mixed-European descent (65%). Approximately one-third (37%) of individuals had antemortem trauma, ranging from healed toes to noses. Manner of death was classified as undetermined for most (75%) of the cases; the second most common classification was suicide (19%). Approximately 70% of the cases were personally identified. Among the identified, 42% were known to have experienced homelessness at the time of their death. Based on the circumstances under which their remains were found, more than one-third (38%) of the unidentified were likely to have experienced homelessness at the time of their death. All the people whose remains were personally identified and who were known to have experienced homelessness also had histories of either mental illness, drug abuse, or both. Approximately half (55%) of the people whose remains were personally identified and who were not experiencing homelessness, had either a documented mental illness, mental degenerative disease, and/or a history of drug abuse.

Synthesis of data extracted from forensic anthropology case files provides fine-grained information regarding a subset of people who were marginalized in life and is potentially useful to public planners. This study suggests that marginalized people are more likely than the average person to become the subject of forensic anthropology cases. From this perspective, the lesions inscribed in the remains of people who become the subject of forensic anthropology casework provide evidence of the types of systematic oppression that they experienced during life. The results also suggest that people who experienced homelessness or were otherwise marginalized at the time of their deaths may also experience a postmortem erasure of identity that is only partially recoverable through the development of biological profiles. Thus, this is a social justice alert to public administration officials and the community at large that not only are people who experience homelessness at risk of a multitude of injustices in life, but they also face injustice in death by the loss of their personal identity and untold stories.

#### Forensic Anthropology, Hawaii, Homelessness



### A132 The Use of Ground Penetrating Radar (GPR) in the Search for Remains of Missing United States Servicemembers

Sabrina C. Ta'ala, MA\*, Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI 96853-5530; Ryan Taira, BA, Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickman, HI 96853

**Learning Overview:** After attending this presentation, attendees will understand the potential benefits and limitations of GPR technology when utilized for the location and recovery of buried human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing knowledge of: (1) the potential applications of GPR to forensic archaeological field problems; (2) specific limitations of this technology, as illustrated in two test cases; and (3) recommendations for critically evaluating and implementing strategies for forensic archaeological GPR use.

Among forensic archaeological remote sensing tools, GPR is arguably the most well-known and the most subject to public misperception, due in large part to its unrealistic depiction in popular media. The technology has great potential; however, the practical application of GPR is complex, and our realistic understanding of its limitations is skewed by the tendency for positive results to be reported, while negative or ambiguous results remain unpublicized.

The Defense POW/MIA Accounting Agency (DPAA), tasked with searching for, recovering, and identifying the remains of missing United States servicemembers from past conflicts, has employed a variety of remote sensing technology for decades. Results using GPR have been equivocal.<sup>1,2</sup> Two of the primary recommendations based on analysis of previous field tests were the need to utilize post-processing software and to conduct further tests in a variety of field conditions. DPAA has recently had the opportunity to perform these recommendations with the acquisition of new equipment: two GSSI UtilityScan® GPR kits, including 350MHz antennae; associated data collection tablets; and RADAN® software. Basic but intensive training on this newly acquired equipment was completed, led by a GSSI representative.

The first case study presented involves data collected at the Manila American Cemetery in the Philippines. Graves of unidentified United States servicemembers exhumed to date at this cemetery have presented challenges involving unexpected placement configurations and depths. The purpose of employing GPR at this cemetery was to assess the potential for the equipment to refine DPAA's exhumation strategy by identifying casket configurations and depths prior to digging. Data were collected over dozens of graves, ten of which were exhumed directly thereafter. Soil composition, casket configuration, and depth were thus confirmed immediately after GPR data collection. Data were examined both in "real-time" and through subsequent analysis using RADAN® software. Results in the Manila American Cemetery were negative. In all but one grave location, GPR was unable to detect anomalies below a depth of approximately 70cm; thus, none of the 1.7 meter<sup>+</sup>-deep steel caskets were detectable prior to exhumation. The likeliest explanation for these results was the soil's high moisture and clay content.

The second case study involves data collected at a large (~70-meter x 30-meter) site on Guadalcanal, Solomon Islands, believed to contain field burials of eight United States servicemembers. The site is associated with a World War II-era sketch map that roughly delineates purported burial locations. The site is slated for excavation in the near future. The purpose of employing GPR in this case was to predict grave locations and thus reduce the amount of block excavation ultimately necessary to recover the remains. High-priority excavation targets would be those in which a correlation is found between GPR-detected anomalies and map-indicated graves. Data were collected in grid format over the entire 70m x 30m site area. Data were examined both in "real-time" and through subsequent analysis using RADAN® software. In this second case, results were ambiguous. While numerous subsurface anomalies were detected with GPR, no clearly defined loci were pinpointed to indicate graves with confidence, and a comparison of the GPR data with the historical map does not present obvious correspondences. The likeliest explanation for these results rests on interference from scattered household trash, rocks, roots, and distortion introduced while managing the data collection equipment over relatively rugged terrain.

GPR technology is complex, a variety of factors can impact results, and data interpretation is not always straightforward. While GPR can help to develop and refine excavation strategies, it cannot necessarily be used as an exclusionary tool. Particularly in forensic archaeological contexts, the possibility of false negatives and false positives must be considered.

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#### Ground Penetrating Radar, Forensic Archaeology, Buried Human Remains



### **A133 The Use of Ground Penetrating Radar (GPR) to Possibly Locate Remains Associated With Surgical Devices**

*Adam Munera\*, George Mason University, Fairfax, VA 22030*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### A134 The Forensic Utility of Photogrammetry in Surface Scene Documentation

Elizabeth Church\*, Boston University, Boston, MA 02118; James Pokines, PhD, Boston University School of Medicine, Boston, MA 02118; Christopher J.H. Ames, PhD, University of Wollongong, Wollongong, New South Wales 2522, AUSTRALIA

**Learning Overview:** After attending this presentation, attendees will understand how Structure from Motion (SfM) can be implemented as a forensically viable photogrammetric technique for 3D documentation of crime scenes, providing a more detailed understanding of evidentiary and environmental relationships in comparison to 2D mapping and eliminating subjectivity in documentation by capturing the scene as a genuine whole. Additionally, this presentation will examine the utility of open-source and commercial photogrammetric software.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the growing literature surrounding standards of accuracy for reconstruction methodology and will help evaluate the adoption of a digital technique.

Applied SfM reconstructs models through image point comparisons. A 3D model is produced from a reference photoset that captures a 360-degree view of the subject, and the software employs triangulation to match specific points, datums, across individual photos. The datums are arranged into a point cloud that is then transformed into the final model. Modifying the point cloud into a final product requires algorithms that adjust the points by building a textured mesh from them. One of the disadvantages of SfM is that the point cloud can be “noisy,” meaning that the program is unable to distinguish the features of one datum from another due to similarities, creating coverage gaps within the meshed images. To compensate for this, the software can smooth portions of the model in a best-guess process during meshing. As commercial software does not disclose the adjustment algorithms, this documentation technique, while very useful in other disciplines that regularly apply SfM, such as archaeology, would fail to meet the standards of the *Daubert* and *Kumho* criteria.

A potential solution to this problem is to use open-source software, which discloses the adjustment algorithms to the user. It was hypothesized that the output of open-sourced software solutions would be as accurate as the models produced with commercial software and with total station mapping techniques. To evaluate this hypothesis, a series of mock outdoor crime scenes were documented using SfM and traditional mapping techniques. The scenes included a dispersed set of plastic human remains and various objects that might reasonably be associated with a crime scene. Ten of these scenes were laid out in 10m x 10m units in a New England forested environment, each grid with a slightly different composition, and then documented using a total station/data logger system and camera. The resulting models were built using PhotoScan® by AgiSoft®, the commercial software, and MicMac/Meshlab for Mac OSX as the open-source comparison software. However, accuracy is only part of the concern; the full utility of any one of the workflows is defined additionally by the overall cost effectiveness (affordability and accessibility) and the visual quality of the final model. Accuracy was measured by the amount of variance in fixed-datum measurements (example, femur length) that remained consistent, while visual quality of the photogrammetric model was determined by the ability to see non-fixed datums (example, a syringe) that moved locations between grids.

While there was variance in the metric outputs between the total station and photogrammetric models, the average total variance from an individual scene, regarding the fixed-datum lengths, fell within 0.635cm. This standard is the recommended measurement accuracy for scene documentation as suggested by “The Technical Working Group on Crime Scene Investigation.”<sup>1</sup> However, the quality of the SfM model was extremely variable, with smaller objects and details being lost entirely. Conditions such as light, ground foliage, and topography were found to effect model quality significantly, as well as the amount of available computing power. There was no issue of losing objects or computing power when mapping by total station and processing the data in AutoCAD®. This research demonstrates that there is potential for SfM to be a rapid, accurate, and low-cost resource for research and forensic investigation, but there are limitations that must be taken into consideration.

#### Reference(s):

<sup>1</sup>. Technical Working Group on Crime Scene Investigation. *Forensic Science Communications*. Volume: 2. Issue: 2 Publication date: April 2000.

#### Forensic Anthropology, Forensic Scene Recovery, Photogrammetry



### A135 Best Practice Procedures for Sampling Differentially Burned Bone for Successful DNA Recovery

Katelyn L. Bolhofner, PhD\*, Texas Tech University, Lubbock, TX 79410; Matthew V. Emery, PhD, Arizona State University, Tempe, AZ 85281; Jane E. Buikstra, PhD, Arizona State University, Tempe, AZ 85287-2402; Laura C. Fulginiti, PhD, Forensic Science Center, Phoenix, AZ 85007; Anne Stone, PhD, School of Human Evolution & Social Change, Tempe, AZ 85287-2402

**Learning Overview:** The goal of this presentation is to offer best-practice procedures for the recovery of skeletal samples from burned human remains to recover DNA useful for identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a predictive model for determining which samples from incinerated skeletal remains are likely to produce the best results for Short Tandem Repeat (STR) testing, mitochondrial genome sequencing, and genome-wide Single Nucleotide Polymorphism (SNP) analysis.

Forensic anthropologists are often asked to evaluate burned human remains resulting from a variety of circumstances. When seeking to determine the identity of individuals found in these scenarios, DNA recovery can provide essential information. However, fragments of burned remains often are not amenable to standard DNA recovery and identification, and it is difficult to predict which samples would be most successful in producing useful results. To maximize resources and minimize the destruction of remains, establishing the conditions under which burned bones are most likely to produce DNA is crucial. By examining variables such as heat-induced color change and type/location of element sampled, the research presented here addresses these issues by offering new standards for the recovery of skeletal samples from burned human remains.

Previous experimental studies have reported that bone changes color in a predictable sequence in response to the duration of exposure to heat. This progression is associated with increased fragmentation and loss of collagen, making the bone increasingly not amenable to DNA preservation and recovery. However, some researchers have argued that DNA can be recovered from even calcined bone. Further complicating the sampling strategy for burned skeletal elements is that many burned remains show variable levels of heat alteration across the body. To assess how different degrees of charring/burning affect DNA recovery, this study obtained and documented 80 samples from 27 fire death and cremation cases. Samples were chosen with the further goal of obtaining statistically significant results from different skeletal elements. For remains that were burned only on the extremities, samples were taken from affected long bones with at least two different levels of burning as well as unburnt tissue ( $n=32$  samples). In cases in which the cranium was burned, where possible the petrous portion of the temporal bone, the cranial vault, and the dentition were sampled ( $n=14$  cases). In severely burned cases in which the remains were calcined, a range of skeletal elements were sampled ( $n=11$  cases).

As this research also explores the degree to which visible markers upon thermally altered human remains can be used to predict DNA preservation, the skeletal samples collected were classified into five levels of burning recorded by Munsell Color Charts (I: well-preserved, unburnt; II: semi-burnt, black/brown; III: black burnt; IV: blue-gray burnt; and V: blue-gray-white burnt). For the dental sample, the published classification system (I: well-preserved, unburnt; II: carbonized, black; III: brown/olive; IV: gray; and V: calcined white) was employed.<sup>1</sup> From these elements, DNA was successfully extracted from 68 samples (including blanks) using two different DNA extraction protocols: one currently used to obtain DNA from ancient remains, and another commonly used in forensic DNA analysis.<sup>2,3</sup>

Overall, skeletal elements belonging to burn levels I through III produced the highest yields. Using the total demineralization forensic protocol, those samples in level V yielded no useful DNA. However, the ancient DNA-based extraction technique resulted in recovery of DNA across all five burn levels, demonstrating that DNA may be recovered from calcined bone in some cases with this method. The parietal bone, tibia, metatarsal, metacarpal, and phalanges produced sufficiently high DNA yields ( $\geq 1$  ng/uL) for STR genotyping and next-generation sequencing analysis. Of these elements, the highest yields were obtained from the parietal bone. Contrary to previously published research, the petrous portion of the temporal produced very low yields, as did the dentition sampled.<sup>4</sup>

The results of this study will further the ability of forensic scientists to target those samples from burned human remains most likely to produce high DNA yields, thus increasing the likelihood of positive identification of individuals in these cases.

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#### Burned Bone, Cremated Bone, Identification



### A136 Putting Life Back Into the Bones: The Symptomatology of Pain and Suffering in the Cyprus Research Reference Collection and Its Relationship to Positive Identification

Jennifer A. Sizemore\*, Red Bluff, CA 96080; Anna S. La Valley, MSc, Reston, VA 20190; Emily Brooks, Thousand Oaks, CA; Xenia Paula Kyriakou\*, Odyssey Fieldschool, Norwich, Norfolk NR5 0DG, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will understand the variety, complexity, and distribution of spondyloarthropathies in a modern Cypriot skeletal population and how the diagnosis and symptomatology of the spine associated with clinical research can reveal information regarding how an individual may have lived with these specific joint diseases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by evaluating the combination of signs and symptoms of spondyloarthropathies as indicators of pain in living individuals and will develop a diagnostic framework for positive identification.

Despite that skeletal remains do not communicate an understanding of pain and suffering during one's life course, per se, the prevalence of spondyloarthropathies and those physical signs of their manifestation warrants that, in combination with clinical research, the human side skeletal remains can achieve, ultimately allowing forensic anthropologists to aim for a more holistic understanding of unknown remains and enable a more detailed antemortem profile of the deceased to be comprised.

The use of pathology to complement positive identification is well known. Diseases can be as important as trauma in reconstructing the antemortem profile of the deceased. While a biological profile is the first step in human identification, disease can also contribute to positive identification. Traditional forensic anthropology relies on skeletal pathology that is abnormal. The rarer the occurrence, the greater likelihood it can be matched with antemortem medical records. However, common conditions, such as diseases of the spine (spondyloarthropathies) can be useful. It is hypothesized that there is a relationship between skeletal signs of disease and symptoms; a relationship that is dynamic and could point toward aspects of pain, suffering, and other possible debilitating reactions that may have affected the daily lifestyle when the individual was alive. It is suggested that a symptomatology of pain can be diagnosed in the presence, distribution, and degree of expression of the skeletal traits relating to spondyloarthropathies. Diseases of the spine are very common in human remains, and their association with symptoms is well reported in the clinical literature. This study seeks to explore and bridge the divide between forensic anthropology and clinical research in this sense and attempt to combine the two in understanding skeletal remains in human terms through the study of osteological traits of disease. The objective of the study is to enable forensic anthropologists to more objectively argue in favor of the human state of skeletal remains providing information that is useful to police investigators and could be related to personal, socio-economic, and cultural perceptions associated with the deceased.

A detailed macroscopic analysis was conducted of 20 vertebral columns of known age and sex from the Cyprus Research Reference Collection. Two methodologies were employed, and an inter-/intra-observer error analysis was conducted to confirm the reliability of the tests. The method recorded diagnostic spondyloarthropathies criteria, such as erosive changes, eburnation, osteophytes, syndesmophytes, and ankylosis. More than 4,000 points of analysis were collected by the end of the study. Overall, the sample population exhibited no significant difference between males and females as well as age. Ninety-five percent of the study sample expressed osteoarthritis as well as a significant overlap of other spondyloarthropathies, such as DISH (10%), AS (20%), combination of DISH/AS (10%), ankylosis (10%), and Schmorl's nodes (30%). However, the additional five percent of the remaining set expressed only a singular spondyloarthropathy. These distributions were then statistically analyzed according to the known demographics of each individual and compared to clinical data assessing possible degree of pain and associated symptoms in living individuals diagnosed with spondyloarthropathies. The results showed that patterns of skeletal signs may be indicative of symptoms, some of which may associate with pain and suffering.

It is concluded that combining osteological traits associated with symptoms of spondyloarthropathies reported in clinical literature may also be deduced for the deceased. These symptoms can and would be viewed by those who knew the individual, as their quality of life would be affected by the spondyloarthropathy. Understanding how these symptoms affect an individual within their everyday life would better prepare a forensic case report for investigators to make a positive identification in the absence of medical records.

#### **Pain and Suffering, Spondyloarthropathies, Positive Identification**



### A137 A Multidisciplinary Investigation of Chronic Animal Abuse: Contributions From Veterinary Forensics and Forensic Anthropology

Jonathan D. Bethard, PhD\*, University of South Florida, Tampa, FL 33620-8100; Martha Smith-Blackmore, DVM, Forensic Veterinary Investigations, LLC, Boston, MA 02116

**Learning Overview:** After attending this presentation, attendees will understand how multidisciplinary collaboration between forensic veterinarians and forensic anthropologists can aid investigations of suspected long-term animal abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that forensic anthropologists can assist forensic veterinarians with analysis of skeletal trauma in suspected cases of chronic animal abuse.

Chronic physical abuse of animals is a concern due to the established link of the progression from animal abuse to inter-human violence. It is well-documented that violence directed toward pets is oftentimes a strong predictor of violence directed toward humans, particularly in single household contexts. In addition, an emerging area of veterinary forensics seeks to differentiate injuries caused by accidents from those termed Non-Accidental Injuries (NAI). Differentiating NAI from accidental injuries may be pivotal for investigations related to suspected cases of animal abuse and subsequent legal proceedings related to the prosecution of such cases. A recent contribution by Tong outlines five characteristics that can be used to classify NAI among dogs: (1) presence of multiple fractures; (2) fractures that occur in more than one region of the body; (3) transverse fractures; (4) fractures that present at a later stage of healing; and (5) multiple fractures at different stages of healing.<sup>1</sup> Due to the involvement of skeletal injuries in establishing the presence of NAI, forensic veterinarians may benefit by consulting with forensic anthropologists familiar with preparing bony specimens for analysis and conducting analysis of skeletal trauma.

In this presentation, the manners in which collaboration between forensic veterinarians and forensic anthropologists can aid investigations of suspected NAI are discussed. A case study is described that resulted in the successful prosecution and conviction of NAI in a domestic dog found near Boston, MA, in 2013. A young dog was found alive on the side of a roadway but euthanized shortly thereafter due to extensive injuries that were documented. During the necropsy, the forensic veterinarian realized that a forensic anthropological consultation would be useful due to the extent of injuries found across the animal's skeleton. During the anthropological examination, multiple healed injuries were described and utilized to conclude that the dog had sustained NAI on multiple occasions. The combination of the veterinary and anthropological data, along with the novel use of DNA technology, resulted in a criminal conviction in 2018. Ultimately, this case study demonstrates that collaboration between forensic veterinarians and forensic anthropologists may be an emerging area of both disciplines.

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#### Veterinary Forensics, Non-Accidental Injury, Animal Abuse



### A138 Evaluating Non-Accidental Injury and Comorbidity in Elderly Decedents Through Macroscopic and Microscopic Patterning of Fracture and Fracture Repair

Donna C. Boyd, PhD\*, Radford University, Radford, VA 24142; Jennifer C. Love, PhD, OCME, Washington, DC 20024

**Learning Overview:** The goal of this presentation is to investigate patterning in fracture and fracture repair in elderly decedents suspected of Non-Accidental Injury (NAI). Antemortem and acute fractures from elderly individuals with suspected NAI are macroscopically and microscopically compared to enhance assessments of diagnostic fracture patterns and fracture healing typical of fatal elder abuse and neglect.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a greater understanding of a number of key comorbidity factors influencing fracture pattern and fracture healing in the elderly. This will ultimately provide evidence (and strengthen court testimony) relating to the determination of accidental vs. non-accidental etiology and timing for fractures in forensic death investigations involving suspicions of elderly abuse and neglect.

Research regarding fracture pattern and fracture repair has not commonly been applied to investigations of suspicious elderly deaths. As with suspicious pediatric deaths, diagnoses of elderly NAI typically rely on fracture type, fracture distribution pattern, evidence of repetitive injury, and inconsistencies between injury pattern and the reported traumatic event.<sup>1</sup> For the elderly, suspicious fracture patterns include long bone, thoracic, and maxillofacial fractures, derived from hitting, grasping, or forcibly restraining these regions.<sup>2,3</sup> However, unlike pediatric NAI determinations, a number of comorbidity factors influence and potentially confuse or conflate NAI in the elderly, leading to difficulty in adjudicating these cases in a forensic setting. Although it is widely recognized, for example, that age-related osteoporosis can significantly increase risk for fracture and post-fracture mortality as well as delay the fracture healing process, the influence of this and other age-related factors on elderly skeletal fracture has not been fully described or documented from a forensic anthropological perspective.<sup>4</sup>

This study presents microscopic and macroscopic evidence for fracture pattern, fracture repair, and factors influencing fracture repair in bone derived from four Radford University Forensic Science Institute (RUFISI) and District of Columbia Office of Chief Medical Examiner (DC OCME) elderly decedents exhibiting suspected NAI. The decedents (both male and female) range in age from 62 to 81 years and exhibit a total of 61 fractures. These fractures are documented through case reports as well as more than 650 micrographs depicting the progression of bone healing and age-related bone disease. These data allow investigation of fracture patterning and repair progression in elderly bone.

Results support the patterning of injury in elderly NAI—the elderly decedents examined here exhibit injuries primarily to their thorax (ribs) and appendages; compromised, delayed, and non-union of antemortem fractures are common and relate to a bone model of anabolic osteoblastic deficiency in the elderly skeleton.<sup>1</sup> Osteoporosis, osteomalacia, and other age-related pathologies are observed as comorbidity factors affecting bone fracture, healing, and post-fracture mortality. Observed fractures differ significantly in their healing morphology, process, and timetable compared to adult and pediatric fractures; for this reason, estimates of time since injury for elderly fractures using current adult fracture stages are inaccurate.<sup>1</sup> Fracture morphology is often indistinguishable across accidental and non-accidental as well as abuse and neglect etiologies; therefore, a consideration of injury patterning, repetition, and context is critical for differential diagnoses of elderly abuse and neglect. Microscopic evaluation of bone fracture morphology is recommended as an integral method for evaluation of these cases.

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#### Non-Accidental Injury, Elder Abuse, Elder Neglect



### A139 An Experimental Investigation of Blunt Force Fracture in the Human Mandible

Mariyam I. Isa, MA\*, Michigan State University, East Lansing, MI 48824; Todd W. Fenton, PhD, Michigan State University, East Lansing, MI 48824; Alexis C. Goots, MA, North Olmsted, OH 44070; Feng Wei, PhD, Michigan State University, East Lansing, MI 48824; Roger C. Haut, PhD, Michigan State University, East Lansing, MI 48824

**Learning Overview:** After attending this presentation, attendees will be informed of the results of experimental blunt impacts to human mandibles.

**Impact on the Forensic Science Community:** This presentation impacts the forensic science community by providing baseline data associating known points of mandibular impact with resultant fracture patterns.

Available literature on mandible fractures can largely be categorized into: (1) clinical studies aimed at evaluating frequencies of fracture types; or (2) impact studies aimed at evaluating fracture tolerances. In clinical studies, the precise loading conditions responsible for fracture patterns are unknown. Conversely, tolerance studies typically provide limited information on fracture patterns. The goals of this study were to conduct impacts to human mandibles at five locations, report on fractures produced in each impact, and describe any patterns that emerged.

The experimental sample comprised 13 intact heads from non-edentulous, unembalmed male cadavers. Heads were placed in an upright position using a previously described procedure.<sup>1</sup> A 1-inch long, 2.5-inch diameter cylinder with a mass of 6.45kg was selected as the implement to simulate a single, clenched fist impact. Mandible impacts were performed at an average velocity of  $8.06\text{m/s} \pm 1.46\text{m/s}$  and input energy of  $216.1\text{J} \pm 73.5\text{J}$ , which produced fractures in all cases. Impacts were delivered to the following locations: midline ( $n=3$ ), anterior body at the canine ( $n=3$ ), mid-body at M1 ( $n=2$ ), posterior body at M3 ( $n=2$ ), and ramus ( $n=3$ ). All non-midline impacts were performed on the left side. Following experimentation, each mandible was resected and macerated, and fracture numbers and locations were assessed. AO Foundation Craniomaxillofacial (AOCMF) standards were applied in assigning fractures to one of nine regions including the left and right condylar processes, coronoid processes, bodies, and angles, and the symphysis.<sup>2</sup>

Peak forces producing fracture showed considerable variation without a clear relationship between impact location and fracture force. Peak forces ranged from 1558.3N to 9669.7N (mean= $3733.0\text{N} \pm 2056.0\text{N}$ ).

Thirteen mandibular impacts produced fractures in six anatomical regions defined by the AOCMF section. No fractures were observed in the coronoid processes or the right mandibular angle.

One key result was that impact location appeared to influence the number of fractures produced. Mandibular body impacts were the only experiments to produce fractures in exactly one location; this was observed in 5/7 cases. In contrast, all ramus and midline impacts generated multiple fractures: fractures were observed at two to three locations in ramus impacts and two to five locations in midline impacts.

Another key finding was that impact location influenced fracture location. Impacts to the left mandible always produced at least one fracture on the left mandible. All three ramus impacts produced impact-side condylar process fractures and one to two additional fractures in the left and/or right mandibular body. Similarly, body impacts consistently produced fractures at or adjacent to the impact site: anterior body impacts generated fractures in the left body; mid-body impacts generated fractures in the left body and angle; and posterior body impacts generated fractures in the left angle. One anterior body impact also produced a right condylar process fracture and one posterior body impact also generated a symphyseal fracture. Midline impacts also produced some consistent results: all three impacts generated symphyseal fractures and unilateral or bilateral fractures of the articular portion of the condyle. One impact also produced mandibular body fractures.

All impacts produced at least one fracture approximately at the impact site. However, cases with multiple fractures exhibited considerable variation in the location of additional fractures. Furthermore, few impact locations produced unique results. Fractures of the mandibular body, condylar processes, and symphysis were observed in impacts to various sites. Only one result appeared unique to an impact site: mandibular angle fractures only occurred in body impacts. These results indicate that when multiple mandibular fractures are present, it is necessary to look to tension and compression features to reconstruct bending direction.

This presentation communicates consistencies and variations in fracture patterns generated in impact experiments to human mandibles. These results contribute a useful comparative sample of known blunt trauma cases for practitioners evaluating mandibular fractures in forensic cases.

*This project was supported by the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect the views of the Department of Justice.*

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#### Blunt Force Trauma, Trauma Analysis, Facial Fracture



### A140 Assessing the Impact of High- Versus Low-Velocity Thoracic Trauma: A Study of Experimental Rib Fracturing Using Juvenile Pigs (*Sus Scrofa*)

Harman Nahal, BSc\*, Simon Fraser University, Dept of Archaeology, Burnaby, BC V5A 1S6, CANADA; Luisa Marinho, MSc, Simon Fraser University, Burnaby, BC V5A1S6, CANADA; Carolyn J. Sparrey, PhD, Simon Fraser University, Surrey, BC V3T0A3, CANADA; Hugo Cardoso, PhD, Simon Fraser University, Burnaby, BC V5A 1S6, CANADA

**Learning Overview:** After attending this presentation, attendees will better understand how juvenile porcine torsos behave under conditions of high-velocity versus low-velocity impact.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing experimental data that informs how the juvenile torso as a structure, rather than bone as a localized material, behaves under different impact velocities.

Forensic anthropologists are often unable to use the same techniques they use on adults to assess trauma on juveniles, due to several differences in bone properties (e.g., structure, elasticity). Rib fractures are one of the most common injuries sustained due to both accidental and non-accidental trauma. When injuries occur from a fall or a motor vehicle collision, the impact will likely result in distinct fracture patterns. However, there has been little experimental data that focuses on the type and pattern of fractures that occur from high-velocity versus low-velocity impact to the juvenile torso. Through an analysis that compares the differences between velocities and anatomical position, forensic specialists will be able to use the findings of this study on bone fracture characteristics to further understand the mechanisms of injury.

This study uses a juvenile porcine model to examine the effects of experimental impact to the torso at two different loading rates: (1) static loading (at a rate of 0.01m/s); and (2) dynamic loading (at a rate of 0.4m/s). Additionally, the torsos will be tested in two distinct starting positions (anterior and lateral) under the two loading rates. A total sample of 12 ungutted juvenile pigs (*Sus scrofa*) aged approximately one week and weighing between 1.5 and 2.4 kilograms were purchased from a local supplier. The total sample was separated into four sub-sample groups, each with three specimens, to assess for differing velocities and different impact positions. The first sub-sample assessed the effects of static loading with impact occurring in an anterior-to-posterior direction. The next sub-sample assessed static loading with the torso being impacted in a lateral position. The remaining two sub-samples assessed the effects of impact in the same two starting positions under dynamic loading. All the specimens were mechanically compressed to 60% of the specimens' torso thickness using the Instron ElectroPlus™ E10000 biomechanical tester. Once compression tests were completed, each specimen was defleshed and the torso was macerated for macroscopic analysis. The sub-samples comprised a total sample of 363 individual ribs.

Macroscopic analysis revealed that of the 363 ribs, only 29 (7.9%) were fractured. Of these 29 fractured ribs, 23 (79.31%) occurred on the right side of the ribcage, while 6 (20.68%) occurred on the left side. Additionally, 17 (58.62%) of the fractures were incomplete and 12 (41.37%) were complete fractures. When comparing fractures that resulted from static loading versus dynamic loading, the results illustrate that 15 fractures (51.72%) were the result of static loading, and 14 fractures (48.27%) were due to dynamic loading. When comparing the two different anatomical positions, 22 (75.86%) fractures occurred when the specimen was impacted anteriorly, while 7 (2.41%) fractures occurred from testing in the lateral position. Further analysis, including the documentation of the specific fracture location on the rib shaft, as well as a thorough characterization of the fracture morphology (macro and microscopically), will enhance the current knowledge the forensic community has on the effects of compressive trauma to the juvenile thorax.

#### Velocity Trauma, Fracture Patterns, Force Velocity Impact

## A141 Factors Affecting Bone Speed of Sound (SOS) in Infants

Miriam E. Soto Martinez, PhD\*, Harris County Institute of Forensic Sciences, Houston, TX 77054; Christian Crowder, PhD, Southwestern Institute of Forensic Sciences, Dallas, TX 75207; Brian Dawson, MS, Baylor College of Medicine, Houston, TX 77030; Urszula Polak, MS, Baylor College of Medicine, Houston, TX 77030

**Learning Overview:** After attending this presentation, attendees will be able to identify characteristics of infant bone that affect tibial SOS measurements.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that normal characteristics of growth and development influence measurements used to infer fracture susceptibility.

The assessment of bone fragility may be pivotal in the diagnosis or exclusion of non-accidental injury as the cause of trauma. However, there is no non-invasive, quantitative method for evaluating skeletal fragility in infants. Such a method could provide substantiated medical evidence of bone fragility or lack thereof in cases of suspected physical abuse. Quantitative Ultrasound (QUS) is marketed as a non-invasive, quantitative technique for evaluating bone quality and is considered a reliable technology for osteoporosis screening in adults.<sup>1-6</sup> Studies indicate SOS, a parameter of QUS, is correlated with bone characteristics related to bone quality.<sup>7-16</sup> Pediatric research suggests that QUS may also be used to evaluate the fragility of pediatric bone, but results are not definitive.<sup>17-28</sup> The purpose of this study is to evaluate the relationship between SOS and volumetric Bone Mineral Density (vBMD) and Bone Volume Fraction (BV/TV) measured by micro-computed tomography. It was hypothesized that SOS would be significantly related to age BMD.

The study sample consisted of 50 (41 term, 9 premature) infants between the ages of 30 weeks gestation (actual or corrected) at birth to one year postnatal at the time of death autopsied at the Harris County Institute of Forensic Sciences. Post autopsy, SOS (m/s) was measured on the anteromedial aspect of the left tibial midshaft using the Sunlight Omnisense 7000S. Subsequently, a Dremel® 4000 rotary tool with a diamond cutting wheel was used to cut a plank (~2mm x 10mm) from the tibial midshaft approximate to the SOS measurement site. Using a Scanco µCT 40 microCT scanner, tibial planks were imaged at 12-micron resolution at a voltage of 55kV and an intensity of 145µA. Analysis of vBMD and BV/TV were performed using the Scanco µCT version 6.1 analysis software with a threshold of 300 and a gauss of 0. One hundred consecutive slices were selected from the approximate center of each plank, and the cortical bone was contoured using a semi-automated method. During analysis, marked Subperiosteal New Bone Formation (SPNBF) was noted on the tibial surface of 23 bone planks.

Multiple linear regression and correlation analyses were used to examine the relationship between age, SOS, vBMD, and BV/TV. Regression results indicate a significant cubic relationship between age and SOS ( $R_{adj}^2=.22$ ,  $F(3, 49)=5.77$ ,  $p=.002$ ); SOS significantly decreased during the first three months of life ( $age^3 \beta=-2.43$ ,  $p=.020$ ), rebounding by 6-7 months of life ( $age^2 \beta=40.00$ ,  $p=.013$ ), and leveling off or decreasing thereafter ( $age \beta=-149.39$ ,  $p=.026$ ). Age also significantly correlated with vBMD ( $r_s=.51$ ,  $p <.001$ ) and BV/TV ( $r_s=.59$ ,  $p <.001$ ). A scatter plot indicated all samples with marked SPNBF were obtained from infants between 0-4 months or greater than 9 months of age. SOS was also significantly correlated with vBMD ( $r=.53$ ,  $p <.001$ ), BV/TV ( $r=.55$ ,  $p <.001$ ), and SPNBF ( $r=-.45$ ,  $p=.001$ ). A model predicting SOS from age<sup>2</sup>, age, vBMD, and SPNBF accounted for 36% of the variance in SOS ( $R_{adj}^2=.36$ ,  $F(4, 45)=7.76$ ,  $p <.001$ ).

Results indicate that SOS is negatively affected by the presence of SPNBF on the infant tibia. SPNBF results from normal growth and development or a trauma response.<sup>29,30</sup> This study's findings suggest that the initial decrease in SOS during the first few months of life is likely due to the presence of SPNBF during this period of growth and development and not a result of decreased BMD. Additionally, results indicate that BMD has a positive effect on SOS. Other characteristics related to bone quality are also likely influencing SOS, as only a portion of the variance is explained by age, BMD, and SPNBF.

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### Bone Mineral Density, Subperiosteal New Bone Formation, Quantitative Ultrasound





### A142 An Analysis of Skeletal Demographics and Traumatic Injuries From the Khmer Rouge Period in Cambodia

Julie M. Fleischman, PhD\*, Harris County Institute of Forensic Sciences, Houston, TX 77054

**Learning Overview:** After attending this presentation, attendees will be acquainted with the osteological analyses that were undertaken at the Khmer Rouge-period mass graves of Choeng Ek in Phnom Penh, Cambodia. This presentation will focus on a sample of crania from the more than 7,000 human remains housed within a memorial stupa (Buddhist shrine) at the site.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing scientific data regarding the demographics and traumatic injuries of the victims of mass violence in Cambodia. While much is known about the Khmer Rouge period from survivors and sociopolitical narratives, until recently, the disinterred human remains had not been scientifically analyzed on a large scale. The crania at Choeng Ek were assessed for demographic characteristics and traumatic injuries to discern whether execution methods were systematic between sex and age groups. The results provide valuable scientific and historical data, although there are limitations.

The Khmer Rouge regime-controlled Cambodia from 1975 to 1979. Conditions were severe and millions perished from overwork, poor health, and violence. Hundreds of thousands were executed and buried in mass graves throughout the country, and many of these graves were exhumed in the 1980s. One such gravesite is the Choeng Ek Genocidal Center (Choeng Ek) in the capitol city of Phnom Penh. Choeng Ek was used by the Khmer Rouge between 1977 and 1979 as the execution and burial location for one of its highest-level detention centers: S-21 or Tuol Sleng. After the overthrow of the Khmer Rouge, 86 of the estimated 129 mass graves discovered at Choeng Ek were exhumed. Although the exact number of individuals buried at Choeng Ek may never be known, today the remains of approximately 7,700 victims are curated within the stupa. This presentation will discuss the osteological analysis of more than 500 crania at Choeng Ek.

The crania were evaluated to determine demographic characteristics and traumatic injury patterns. For each cranium, the following data were assessed and recorded: date of analysis, identification number, sex, ancestry, age at death, traumatic injuries, and any pathologies or anomalies. Sex and ancestry were evaluated using morphoscopic characteristics and age at death was evaluated using cranial suture closure, specifically the maxillary sutures. Regarding the skeletal injuries, antemortem, peri-mortem, and postmortem trauma or damage was recorded. If traumatic injuries were present, the following information was documented: location (i.e., specific cranial bone(s)), timing, mechanism (i.e., blunt, sharp, high-velocity projectile/gunshot wound, or indeterminate), type of fracture (i.e., depressed, (linear) radiating, concentric, or other), whether the injury represented a direct impact, and the cranial region affected. All 508 crania were photographed, and some were radiographed.

Results indicate that the majority of the 508 crania were estimated to be male (82.9%) and young adults (68.3%) between the ages of 20 and 35 years old. When ancestry could be assessed, all the individuals were Asian. Therefore, the majority of individuals analyzed were young adult males of Asian ancestry. Peri-mortem trauma was present on 311 crania (61%), with 179 (58%) having discernable impact locations. Blunt force injuries (87%) were the most common mechanism of trauma and the basicranium (53%) was the most frequently impacted region. When the mechanism and location of traumatic injuries were evaluated by sex and age-at-death categories, no statistically significant differences were found, indicating that all victims with peri-mortem trauma were subjected to similar execution methods, regardless of their age or sex.

The available skeletal trauma results are consistent with the historical accounts of how the Khmer Rouge executed individuals at Choeng Ek. According to eyewitness accounts, Khmer Rouge victims were generally executed by being struck on the head or base of the neck (i.e., the basicranium) with a hard object (i.e., blunt force trauma). Thus, the results of this research suggest that the demographics and traumatic injuries of the Choeng Ek crania complement the historical narrative of the Khmer Rouge period. Ultimately, these remains stand as a testament to the violence that transpired as well as a solemn memorial to those who perished. The analysis of human skeletal remains is imperative for the construction of a more comprehensive understanding of the Khmer Rouge period in Cambodia.

#### Skeletal Trauma, Khmer Rouge, Cambodia



### A143 Diversity and Inclusion in Forensic Anthropology: Where We Stand and Prospects for the Future

Sean D. Tallman, PhD\*, Boston University, Boston, MA 02118; Cate E. Bird, PhD, International Committee of the Red Cross, Washington, DC 20036

**Learning Overview:** After attending this presentation, attendees will better understand how forensic anthropologists currently perceive diversity and inclusion in the Anthropology Section.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of diversity and inclusion, and how they can benefit the forensic anthropology community. Additionally, this presentation will outline the development of the Diversity and Inclusion Committee within the Anthropology Section, as well as its primary objectives, survey results, and proposals of how individuals and institutions can increase diversity at multiple levels within applied and academic contexts.

Diversity and inclusion has proven to be an asset for individual and group performance, growth, development, and success in business and academia through principles of flexibility, equifinality, and respect for differences. Research demonstrates that diverse communities or workforces result in sustained competitive advantages through increased innovation and creativity. In 2017, the President of the AAFS Anthropology Section created an ad hoc committee to critically explore the current state of diversity within forensic anthropology. In 2018, this ad hoc committee was enthusiastically changed to a permanent Diversity and Inclusion Committee by voting members (AAFS created a similar Diversity and Outreach Committee in 2015 to develop comprehensive strategies to support diversity and promote collaboration, professionalism, and integrity). Primary objectives of the Committee include assessing diversity-related concerns of the Section, promoting inclusiveness, acting as an advocate for underrepresented individuals in forensic science, and promoting diversity-related resources for all Section members. This venture importantly parallels committees formed by closely aligned professional organizations, including the American Association of Physical Anthropologists and the Society for American Archaeology, and thus has the advantage of observing how diversity and inclusion movements in similar organizations have successfully resulted in innovation, renewal, and growth.

To evaluate the current state of diversity and inclusion in forensic anthropology, the Committee created and distributed an Institutional Review Board-approved survey to all members of the Anthropology Section (University of South Florida proposal #00032513). Of the 220 participants, ages range from 18-85+ years, with most being 25-44-year-olds (58.8%) who hold various degrees/certifications, including BA/BS (13.3%), MA/MS (26.1%), PhD (28.0%), MD/PhD (12.8%), and PhD/Diplomate status in the American Board of Forensic Anthropology (19.9%). Of the respondents, only 30.9% self-identify as a member of an underrepresented group, including African American, Native American, Asian, Hispanic/Latinx, LGBTQ+, and/or disabled. Heterogeneity is believed to be the greatest at the undergraduate level, with diversity decreasing in vertical movement through graduate, postdoctoral, and/or professional statuses. Overall, the majority (63.9%) of respondents believe that forensic anthropology exhibits little diversity; this lack of diversity is considered problematic to 73.4% of respondents, while 84.3% believe the Section should do more to recruit and maintain diverse forensic anthropologists.

While 90.6% of respondents feel accepted within the Anthropology section, discrimination is an issue: 24.0% of respondents have experienced discrimination, while 46.3% have witnessed discrimination in the Section. Issues of discrimination, disrespect, and exclusion can have a particularly negative effect on our community, as it can lead to attrition of those with diverse backgrounds and perspectives. Furthermore, these figures are especially worrisome since 59.9% of respondents do not know how to report incidents of discrimination within the Section.

Like comparable endeavors in other scientific and non-scientific contexts, the Committee contends that diversifying forensic anthropology through outreach and inclusion of persons with unique backgrounds and life experiences will ultimately benefit our science. While the Committee recognizes a strong tradition of scientific integrity in forensic anthropology, our community's homogeneity, exclusivity, and competitive nature may make us blind to assumptions in our scientific interpretations. The Committee believes diversity and inclusion in research and practice permits greater innovation, and the propensity to critically examine our assumptions, particularly as they relate to the complex and dynamic populations that we serve. This study demonstrates that our scientific community faces two major hurdles in this venture: *recruitment* and *retention* of diverse members. Through thoughtful and concerted efforts to value diversity during graduate school selection and hiring processes, to support mentorship programs, and to foster an environment of respect and acceptance in our Section, the Diversity and Inclusion Committee believes forensic anthropology can greatly improve and serve as a successful model within the forensic sciences.

#### Diversity and Inclusion, Forensic Anthropology, AAFS



### A144 Increasing Graduate Student Diversity in Forensic Anthropology Through Research

Kate Spradley, PhD\*, Texas State University, San Marcos, TX 78666; Olivia A. Jones, Texas State University, San Marcos, TX 78666

**Learning Overview:** After attending this presentation, attendees will better understand how a diverse research agenda or applied focus can serve to increase diversity in forensic anthropology graduate students and professionals. This presentation focuses on published forensic anthropological research and explores diversity in research as one concept for attracting and retaining underrepresented, socially defined racial and ethnic groups.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing attention to a perceived lack of diversity in development of forensic anthropological methods and validation studies.

Diversity and inclusion are at the forefront of Science, Technology, Engineering, and Mathematics (STEM) recruitment, including the forensic sciences. As evidence of commitment to diversity and inclusion, the American Academy of Forensic Sciences (AAFS) began the process of creating a Diversity Committee in 2016. In 2018, The National Institute of Justice (NIJ), a major funding source for the forensic sciences, published a report, “The Importance of Diversity and Inclusion in the Forensic Sciences,” citing that diversity of race, ethnicity, gender, socioeconomic status, and background in the workplace drives innovation, creativity, and enhances problem solving.<sup>1</sup> The NIJ report refers to the “leaky pipeline,” indicating that while diversity is present in K–12<sup>th</sup> grade, it declines incrementally at the undergraduate level through graduate school, resulting in a lack of diversity in workplace and in academia.

Diversity has a broad meaning, including diversity of thought, religion, sex, gender, gender identity, sexual orientation, age, social class, physical ability, and race and ethnicity. The AAFS currently does not request information about diversity in membership requirements; however, in a 2012 statement in the *Academy News*, the President-Elect discussed the lack of African Americans and Hispanics in the forensic sciences and noted that “looking around the room at the scientific or social functions held during the annual meeting reveals that we are a largely Caucasian organization”.<sup>2</sup> Many forensic science fields require advanced degrees; however, because of the “leaky pipeline,” forensic anthropology, like other disciplines, lacks diversity in graduate students, applied professionals, and academics.

Within the Department of Anthropology at Texas State University (TSU), graduate student applications from underrepresented racial and ethnic groups have increased over the past ten years. These applicants indicate that faculty research and applied programs with a focus on underrepresented groups makes the graduate program attractive. To document diversity in research within forensic anthropology, a literature review was performed focusing on the *Journal of Forensic Sciences*, the flagship journal of the AAFS. A keyword search using “forensic anthropology” was initiated and refined using the search terms “ancestry” and “race” from 2006–2018. Each article was reviewed for the population groups used (e.g., Black, Hispanic) and each group was recorded, using the same terminology within the publication. Although the search terms focused on ancestry, the returns included overlap with sex, stature, and age estimation, as well as other foci and included 199 publications.

The majority of publications (81) focused on one group, 59 on two groups, 26 on three groups, 15 on four groups, and 18 on five or more groups. A total of 22 publications focused on White only, 34 European only (with 6 using White North Americans), and 23 Black and White only. Of the papers focusing on White individuals only, 14 focused on developing new methods or validating methods. Papers focusing solely on Hispanics, Central Americans, Western or Eastern Europeans, Southeast Asians, or South Africans typically focused on generating population-specific techniques lacking in the literature.

The majority of recent skeletal collections in the United States are predominantly White, followed by Black, with more males than females. However, these collections also contain individuals that are Hispanic, Southeast Asian, and Middle Eastern, in addition to other groups, although in lesser numbers. While travel outside the country may be cost prohibitive without grant funding, data sharing, and data from Computed Tomography (CT) scans obtained from hospitals and health science centers can also increase data from underrepresented groups. Incorporating diverse groups into the development of new methods and validation studies should be a priority to improve practice and increase diversity in graduate students and professionals within our field.

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#### Forensic Anthropology, Diversity, Graduate Students



### A145 Perspectives on Diversity in Skeletal Collections

*Allysha P. Winburn, PhD\**, University of West Florida, Pensacola, FL 32514; *Antaya L. Jennings, BA\**, Pensacola, FL; *Dawnie W. Steadman, PhD*, University of Tennessee, Knoxville, TN 37996; *Elizabeth A. DiGangi, PhD*, Department of Anthropology, Binghamton, NY 13902

**Learning Overview:** After attending this presentation, attendees will better understand the many complex factors influencing living individuals' willingness to donate their remains for forensic scientific study. For communities of color, factors may include prioritizing justice for the living over study of the dead, informed by cross-generational, community-level memories of historic scientific misuses of human remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by portraying perspectives that are infrequently represented in forensic science research: those of living donors from communities of color.

The development, testing, and refinement of forensic anthropology methods are contingent on the analysis of donated skeletal collections representing documented individuals from various ancestral backgrounds. In the United States, forensic anthropologists must have an adequate understanding of modern United States skeletal variation to construct the biological profiles that enable personal identifications. However, the few existing United States collections of donated, fully documented skeletons do not currently reflect modern United States ancestral demography. Specifically, European American individuals are overrepresented in these collections, while African American, Asian American, Native American, and Hispanic individuals are underrepresented. Further, where the remains of people of color are present in fully documented collections, they may represent medical examiner donations whereby families were unable or unwilling to claim the remains. From a collections perspective, such donations suffer from the lack of documentation other than sex, ancestry, and perhaps age. While medical examiners can legally consent to donation, families may or may not have been told that donation was the final disposition, rather than burial or cremation. Therefore, some institutions have eliminated this practice (e.g., the University of Tennessee's Forensic Anthropology Center).

In line with the above, there are ethical considerations when analyzing remains of individuals who may not have explicitly consented to the use of their remains in this fashion. While consent standards followed by Institutional Review Boards do not include research on the deceased, this does not mean that handling another individual's remains is without ethical concerns. Different cultures and individuals have varying perspectives on what happens after death; some may believe that the remains themselves can be harmful to researchers, or they may simply desire that their bones be left alone to decay as nature intended. While practitioners have a desire for diverse collections, balance must be achieved with the beliefs and customs of the people who are most underrepresented in these samples.

From the forensic anthropological perspective, ancestral disparities in skeletal collections are a scientific shortcoming to be resolved. Practitioners know that in absence of diverse, documented skeletal collections on which to base their methods, their understanding of modern United States skeletal variation will suffer, and correct identifications may decrease. However, for people of color considering whether to donate their remains, the issue is more complicated. Past biological anthropologists had a long history of building scientific knowledge quite literally on the backs of non-consenting Native Americans, African Americans, and other individuals of color. The ancestral disparities in United States skeletal collections likely result not only from a lack of conversation between scientists and people of color, but also from a deep, historical mistrust between these communities. Further, in a nation where it still must be asserted that African American lives matter, communities of color must prioritize numerous other issues above studying the remains of individuals who are already deceased. Education level, religious preference, and socioeconomic status may also influence donation decisions, along with the fear of perceived complicity with a "white agenda." These issues are further complicated by the fact that social and medicolegal injustices may feed back into one another, potentially translating to ancestral disparities in whose remains are identified and who becomes a "cold case."

Forensic anthropologists use knowledge of human skeletal variation to resolve medicolegal cases, ultimately leading to identifications for the dead and justice for the living. When they use this expertise to inform broader conversations about scientific ethics and social justice, they have the potential to benefit not only the medicolegal system but also United States society as a whole. While forensic anthropologists may never amass skeletal collections that truly match United States ancestral demographics, this is a topic worth exploring—in all its complexities and from multiple, sometimes conflicting, perspectives.

*Financial support for this work was provided by the University of West Florida Office of Undergraduate Research Summer Undergraduate Research Program.*

### Race And Ancestry, Body Donation, Ethics



### A146 Why Are There So Many Women in Forensic Anthropology? A Critical Evaluation of Gender Politics in Forensic Anthropology

Marin A. Pilloud, PhD\*, University of Nevada, Reno, Reno, NV 89557-0096; Nicholas V. Passalacqua, PhD, Western Carolina University, Cullowhee, NC 28723

**Learning Overview:** After attending this presentation, attendees will understand the current position women hold in the field of forensic anthropology in terms of student enrollment, professional positions, organizational membership status, and leadership roles. Additionally, sexual harassment and discrimination within the discipline are addressed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing needed information on a long-going discussion within forensic anthropology as to the number of women in the discipline, primarily in comparison to men. It will further outline issues surrounding sexual harassment and discrimination and provide suggestions for a path forward.

While attention is constantly drawn to the number of women currently pursuing and practicing forensic anthropology, there is little data to investigate the relative changes over time in the gender of individuals in the discipline. Passalacqua and Pilloud recently addressed this issue and showed that females greatly outnumber males in membership within the Anthropology section of the American Academy of Forensic Sciences (AAFS), particularly in the student and trainee affiliate categories.<sup>1</sup> Further, Passalacqua and Pilloud demonstrated that men have traditionally outnumbered women in disciplinary leadership positions (e.g., section chair, Diplomates of the American Board of Forensic Anthropology, and recipients of the T. Dale Stewart award), although there is a shifting trend over the past decade to favor more women in these positions.<sup>1</sup>

To further explore gender differences, grant funding and tenure-track positions in forensic anthropology were evaluated. Grant funding was assessed using the National Institute of Justice list of graduate research fellowships since 2000. Among those with forensic anthropology as a focus of research, eight fellowships were awarded to women and six to men. This number is surprisingly comparable despite the larger number of female student affiliates in the AAFS anthropology section. Further, men were on average awarded more money than were women. The number of tenure-track faculty positions for forensic anthropologists were evaluated at PhD-granting universities. As programs have grown and faculty have retired, there has been a shift toward more women in these positions that were previously held by men.

While trends are moving toward more inclusion of women in forensic anthropology, there is still a pervasive gender bias and work to be done in combating sexual discrimination. Such biases have been well documented in the sciences in the: peer review process, election to leadership roles, awarding of research and career grants, and hiring and promotion. In addition to pervasive sexism hindering progress in their careers, women may also be subjected to sexual harassment. Such harassment can occur in places of employment, during fieldwork, in the classroom, or at professional conferences.<sup>2-4</sup>

Despite the growing number of women forensic anthropologists, it still may be difficult for them to achieve leadership positions or gain respect from male colleagues. In the face of such challenges, women may be more likely to leave the discipline or to seek positions with more flexibility, less authority and responsibility, and lower salary. Further, the question itself, “Why are there so many women in forensic anthropology,” reinforces these gendered stereotypes by putting the onus on women to explain why they are drawn to forensic anthropology, with the assumption that they should not be.

To improve gender equality and overall diversity within the discipline, there needs to a shift in how this problem is addressed, with a focus on retention and promotion of women within the discipline. Solutions could include making workplace accommodations such as: flexible hours, workplace sexual harassment and discrimination training, transparency on equal wages, and clear policies and repercussions for sexual harassment and discrimination. Within the discipline, there should be a move to double-blind peer reviews, and professional organizations could provide outlets for women to gain additional mentoring as they navigate their careers. Finally, professional organizations must take a strong and clear stance against sexual discrimination and harassment that is well articulated and contains clear consequences for violations. Through such a concerted effort, forensic anthropology is poised to serve as an example for biological anthropology and the forensic sciences in promoting gender equality.

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#### Gender Equality, Sexual Discrimination, Sexual Harassment



### **A147 Recruitment Strategies From the Increasing Diversity in Evolutionary Anthropological Sciences (IDEAS) Program of the American Association of Physical Anthropology (AAPA)**

*Phoebe R. Stubblefield, PhD\*, University of Florida, Gainesville, FL 32610; Susan C. Anton, PhD, New York University, New York, NY 10003*

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**Learning Overview:** After attending this presentation, attendees will understand the history and the mode of operation of the IDEAS program from the perspective of one of its faculty mentors and how to assess the applicability of IDEAS or alternate models to diversity recruitment efforts within the anthropology section.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing strategies for diversity recruitment for those forensic sciences that have a broad foundation within academia as opposed to professional settings but see few entering forensic careers.

Toward the effort to increase diversity within the Anthropology Section of the American Academy of Forensic Sciences (AAFS), this presentation is a description of an existing program within one of our sister organizations. Derived from the observations of an IDEAS faculty mentor, this presentation explores the strengths and weaknesses of the IDEAS model and proposes development and application of a recruitment program in the Anthropology Section. The IDEAS program, which was developed by Susan Antón and Ripan Malhi (Principal Investigator and Co-Principal Investigator, respectively, NSF award #1516939) addresses the low representation of first-generation college students and/or members of underrepresented traditional minorities in physical anthropology graduate programs and careers, in comparison to numbers observed in undergraduate majors and physical anthropology courses. Funded since 2016, IDEAS operates through a recruitment and network-building process targeting faculty, graduate students, and undergraduates from underrepresented groups. As a longitudinal program, implementation of IDEAS began with the creation of a Committee on Diversity (COD) within the AAPA, which in turn led to a network of faculty committed to diversity recruitment. The two primary factors that affect employment of an IDEAS-like model include financial support and faculty investment across temporal, professional, and social levels. External financial support allowed the COD faculty to implement a strategy that targets the departure of talented undergraduates from physical anthropology careers. Additional support comes from the AAPA in the form of connection to its undergraduate research symposium and support from AAPA leadership.

At a minimum, operating a diversity recruitment strategy requires a longitudinal perspective, including: (1) faculty or professionals drawn from multiple generations within the organization; and (2) a multi-year (strategic) plan of activity. An assessment program that examines the effectiveness of the model and tracks changes in diversity requires clear and actionable goals for the recruitment program. Results, products, and deficits from the IDEAS process will be presented with the goal of developing a feasible recruitment strategy for relevant sections of the AAFS.

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**Diversity, Recruitment, Forensic Anthropology**



### A148 The Morphology of Intersectionality: Discordance Between Ancestry Estimates and Social Identifiers

Chaunesey Clemmons, BA\*, Texas State University, San Marcos, TX 78666

**Learning Overview:** After attending this presentation, attendees will understand the discordance between the identity formed by ancestry estimation and the application of this identity to a sociocultural label used in forensic anthropological contexts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showcasing the importance of the intersectionality of the terminology used in instances of forensic identification.

This study underscores the importance of forensic anthropologists fully considering the intersectional nature of identity. Identity is dynamic, in that for a singular individual, it involves a biological identity, a self-identity, and an identity imposed on the individual by public perception. Biological identity can be recognized anthropologically as ancestral estimations, while self-identity aligns with how the individual views him/herself in accordance with their society and culture (social identifiers), and public perception is how others view the individual through the lens of their sociocultural experiences. Social identifiers can include a variety of demographic categories, many of which include physical representation. This presentation will focus on those used by the United States census, which are the racial and ethnic classifiers used in everyday terminology to describe a person's physical identity. In the case of an unidentified deceased individual, one goal of the forensic anthropologist is to generate a biological profile, part of which informs on ancestry (estimation of most likely geographic origin), to narrow down the possible identity of the unknown individual. Yet, forensic anthropologists are rarely, if ever, the authority that assigns a personal identification.<sup>1</sup> Because of this we must relate our ancestry estimate to currently accepted social identifiers.

In this presentation, data from a sample of 13 (11 female, 2 male) living self-identified Biracial individuals demonstrates a lack of agreement between ancestry estimates and social identifiers. Methods employed to estimate the ancestry of  $N=13$  included current dental morphological quantitative methods following Edgar and rASUDAS, and biological distance analysis.<sup>2,3</sup> Results estimated the majority of the individuals to be European, which in the United States sociocultural context translates to a categorization of White. This study showcases that for a sample of Biracial individuals, the methods used were unable to correctly identify them on any level (biological, self, and public) and suggests that forensic anthropological analysis of the biological identity has the potential to hinder identifications. Forensic anthropologists operate within a medicolegal context; therefore, it is important to understand how our scientific interpretations translate to our sociocultural understanding of identity.

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**Ancestry Estimation, Social Identifiers, Biracial Sample**



### A149 A Push for Trans-Inclusive Language in Forensic Sciences

Fatimah Bouderdaben\*, Spring, TX 77388

**Learning Overview:** After attending this presentation, attendees will: (1) understand the extent and urgency of current trans-exclusive language regarding the forensic sciences; and (2) have the tools necessary to implement trans-inclusive language in everyday practice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the inherent and often unintentional trans-exclusive language that is consistently overlooked by practitioners. This presentation will present data and propose methods to make our discipline more trans-inclusive in both theory and practice.

It has been established in the anthropological community that gender and sex are disparate classifications of an individual that present a tangled web of biological, social, and cultural identity.<sup>1</sup> Yet, the academic literature within the forensic sciences itself is still trans-exclusive. According to the National Coalition of Anti-Violence Programs, most victims of hate violence homicides (72%) in 2013 were transgender women.<sup>2</sup> A study conducted by the American Foundation for Suicide Prevention revealed that 41% of transgender individuals have attempted or committed suicide.<sup>3</sup> Based on the published statistics, it is highly likely that forensic scientists will encounter individuals who identify as transgender. For this reason, when presenting work in a forensic and/or applied setting, the researcher should take care to not mis-gender unidentified victims. Such error could lead to inaccurate reporting, introduce bias, and/or result in an inappropriate representation of how the decedent may have identified.

This study hypothesized that gendered language would be commonly used in forensic academic writing when referring to unidentified remains and sex identification/estimation. To test this hypothesis, forensic academic articles were searched through the Criminal Justice Abstracts database accessible by all Texas A&M University students. Using a variety of keywords to pull as many articles relating to the topic of unidentified remains and sex identification/estimation as possible, a total of 611 articles were evaluated. After reading the 611 abstracts, only 105 of them were considered topical and were read through completely to be marked for the use of trans-exclusive language. For the purposes of this research, trans-exclusive language was defined as “language that gendered an unidentified body” or “language that confused the terms sex and gender.”

Of the 105 academic papers reviewed, approximately 49% of the articles reporting on unidentified remains utilized gendered language, as denoted using pronouns such as he or she, when referring to the remains, even though the gender was still unknown. Approximately 93% of articles dealing with sex identification/estimation would use sex and gender interchangeably when gender was mentioned. The high rates of error could be easily addressed by adopting gender neutral reporting in the forensic field. While the goal of the forensic anthropologist working on an unidentified remains case would be to estimate biological sex, to effectively create a profile, the anthropologist would need to respect the possibility that the unidentified individual could be transgender. Since gender cannot be determined from remains alone, implementing gender neutral language would keep the possibility of the remains belonging to a transgender individual a constant in the mind of the investigator and could greatly decrease the probability of the unidentified individual being mis-gendered. Therefore, gender neutral language will be both beneficial to the forensic science community as well as the individual being identified.

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#### Transgender, Gender Neutral, Forensic Reporting



### B1 Costal Cartilage as an Alternative Source for DNA Typing in Personal Identification

*Marcin Tomsia, St. legionów Polskich 32/46, Olkusz, woj.malopolskie 32-300, POLAND; Rafal Skowronek, Department of Forensic Medicine in Katowice, Medyków 18 Street, Katowice, Upper Silesia 40-752, POLAND; Michal Szczepanski, Medyków 18, 40-752 Katowice, Katowice, Silesia, POLAND; Christian Jablonski, Medical University of Silesia, Medyków 18, Katowice, Silesia 40-752, POLAND; Gulnaz T. Javan, PhD\*, Alabama State University, Forensic Science Program, 915 S Jackson Street, Montgomery, AL 36104*

**Learning Overview:** After attending this presentation, attendees will understand how to use the costal cartilage as an alternative source of sample for DNA typing in personal identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that the costal cartilage can be valuable evidence for DNA typing.

In decomposed bodies, bones and teeth are the most reliable sources of nuclear DNA suitable for PCR typing of STR loci. However, DNA extraction from such kinds of specimen requires long and painstaking laboratory work and the final DNA yields are often extremely small. Costal cartilage as an alternative source of nuclear DNA in decomposed human remains has been evaluated. Cartilage is a specialized type of connective tissue formed by cells, the chondrocytes, lying in an amorphous matrix rich in collagen and elastic fibers. It can be surmised that this matrix may act as a physical barrier protecting DNA from chemical breakdown. Moreover, cartilage is lacking in blood vessels. For this reason, chondrocytes may be less prone to degradative factors such as microorganisms. Cartilage is very well preserved while the skin around the rib has a marbled appearance characteristic of the bloat stage of decomposition. Samples were collected from: (1) murder scenes where the corpse was found in a grave (2 cases, PMI = 2 to 4 years); (2) suicide scenes where the corpse was found in the forest and in flats (30 cases, PMI = 3 months to 3 years); (3) conflagration scenes where the corpse was found in the rubble (3 cases, PMI = 1 day to 7 days); and (4) rockfall in a coal mine (6 cases, PMI = 1 day to 67 days).

In this study, decomposed tissues were removed from the rib sample followed with pre-cleaning in sterile water twice and a final wash with 70% ethanol. A slice piece of costal cartilage was generated by using a surgical blade and incubated in 300 µl water, 300 µl incubation buffer and 20 µl proteinase K at 56° C in 1.5 ml micro-centrifuge tube until the sample was completely resolved. Column technology was used to isolate DNA from the supernatant. The final volume of DNA solution was 50 µl. Purified DNA was measured. The degree of DNA degradation was low and ranged between 0.7 and 1.1. Multiplex PCR was performed with the thermal cycler and the amplification products were separated by capillary electrophoresis. Electrophoretic data was analyzed using the software complete profiles of all 23 autosomal STRs and gender-specific amelogenin X and Y was obtained by using the authors' proposed technique.

In conclusion, the authors demonstrated that the costal cartilage could be a designate specimen for DNA isolation from human decomposed body especially in those cases where the corpse was discovered several years after death. Furthermore, DNA isolation from costal cartilage was simple, fast, less expensive, and no freezer was required. Regardless of the appearance and morphological state of the cartilage, a complete genetic profile was obtained in all cases. Costal cartilage sample should be standard source for isolating high quality and quantity genomic DNA from decomposed bodies.

#### Costal Cartilage, DNA Typing, Short Tandem Repeat



## B2 Population Analysis and Forensic Utility of Sequence-Based X-Chromosomal Short Tandem Repeat (X-STR) Loci

Lisa Borsuk, MS\*, National Institute of Standards and Technology, Gaithersburg, MD 20899; Toni M. Diegoli, PhD, Beltsville, MD; Katherine B. Gettings, PhD, National Institute of Standards and Technology, Gaithersburg, MD 20899; Becky Steffen, MS, National Institute of Standards and Technology, Gaithersburg, MD 20899; Kevin Kiesler, MS, Gaithersburg, MD 20899; Peter M. Vallone, PhD, National Institute of Standards and Technology, Gaithersburg, MD 20899-8314

**Learning Overview:** After attending this presentation, attendees will learn about sequenced-based variation at seven X-STR loci, representing the four X chromosome linkage groups, sequenced from over one-thousand United States population samples with a commercially available sequencing assay. Attendees will understand how this sequence-based diversity compares to the length-based diversity of X-STR loci.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by providing additional information regarding forensically-relevant X-STR loci. Specific impacts include facilitating forensic practitioners' understanding of the performance and informational gains obtained through sequencing X-STR loci. The sequence-based allele and haplotype frequencies in four U.S. population groups will assist with the adoption of sequence-based X-STRs by the forensic practitioner.

X-STR markers are recognized as useful tools to supplement kinship testing in the forensic setting. Numerous studies of allele and haplotype frequencies based on traditional length-based analyses of these loci have been reported in the literature for various population groups. More recently, new technologies capable of providing sequenced-based information with a higher level of marker multiplexing have been investigated for characterization of forensic loci, including X-STRs. The details of sequencing and analysis of seven X-STRs in U.S. populations will be presented.

The National Institute of Standards and Technology (NIST) U.S. Population Sample Set consists of 1,036 unrelated individuals (1,032 male, 4 female) with four population groups represented: African American ( $n=342$ ), Asian ( $n=97$ ), Caucasian ( $n=361$ ), and Hispanic ( $n=236$ ). These samples have been sequenced using the MiSeq FGx Forensic Genomics System, including the ForenSeq DNA Signature Prep Kit, which targets important STR markers commonly used for human identification and relationship testing.<sup>1</sup> Seven X-STR loci are included in this assay: DXS10135, DXS10074, DXS7132, DXS10103, DXS7423, DXS8378, and HPRTB, with at least one marker representing each of the four linkage groups found on the X-chromosome.<sup>2</sup> The core repeat region as well as flanking region variation was assessed with a customized bioinformatic approach. This approach also detected two additional X-STR loci (DXS10148 and DXS8377) which are sequenced with the assay but not reported in the associated Universal Analysis Software (UAS). These two 'extra' loci are being evaluated for potential inclusion in the population set.

Sequence-based allele and haplotype frequencies along with other relevant population genetic parameters for each population group will be presented. Results from this study will be compared to allele calls and frequencies derived from previous analyses using length-based methods.<sup>3</sup> The magnitude of the increase in the number of unique alleles and haplotypes will be presented in the context of potential gains in discriminatory power between the methods. The information provided in this study will serve to facilitate the application of sequence-based methods to X STR profiling in the forensic setting. The sequence data will be made publicly available at NCBI STRSeq X-Chromosomal STR Loci BioProject accession PRJNA380348.<sup>4</sup>

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### X-STR Loci, Sequencing, United States Population



### **B3 A Novel Collection Method That Improves Direct DNA Amplification From Various Bloodstained Porous Materials**

*Jamie D. Fredericks, PhD\**, Eastern Kentucky University, Richmond, KY 40475; *Stephen Lipic*, Eastern Kentucky University, Richmond, KY 40509; *Lucille Giordullo*, Richmond, KY 40475

**Learning Overview:** The goal of this presentation is to demonstrate the effectiveness of a novel collection method, which utilizes FTA™ Elute cards to significantly improve direct genotyping from a variety of bloodstained materials.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing practitioners with a simple and effective collection method that improves the quantity and quality of short tandem repeat (STR) loci directly amplified, while also reducing the time and cost of DNA analysis.

The demand for DNA testing to solve crimes and assist in the identification of victims from mass disasters and terror attacks continues to grow and has contributed to an ever-increasing backlog of samples that require analysis.

Direct Polymerase Chain Reaction (PCR) amplification has focused on genotyping samples from the source, avoiding the time-consuming extraction process attributed to traditional methods. In addition to reducing the cost and time it takes to generate a DNA profile, direct PCR analysis also reduces the amount of DNA lost from sample transfer during the multi-step purification process and minimizes handling, decreasing the risk of contamination.

Directly profiling DNA from its source may not always be possible. Biological evidence can be found on any inanimate object, many of which cannot be sectioned to fit into a PCR tube or may require specialized and expensive equipment that is not readily available to the laboratory. Recovering DNA from trace amounts of blood deposited on porous materials can be problematic. Biological samples can become embedded within the material's porous matrix, potentially reducing the amount of DNA recovered. In addition, samples collected may contain known inhibitors that could impede the PCR process. Hemoglobin from whole blood, indigo dye found in jeans, and humic compounds found in soil have all been shown to inhibit the PCR.

FTA™ Elute cards are traditionally used to store and preserve blood and saliva samples that are typically provided by a donor. Their ability to allow direct amplification of DNA that has become embedded within its matrix has become an invaluable tool to the medical and forensic communities. FTA™ Elute cards contain a chaotropic salt that can lyse cells and keep proteins tightly bound to its matrix while DNA is eluted and made available for amplification. This project investigated a novel application of FTA™ Elute cards in their ability to directly profile minute amounts of blood that have been deposited on different porous structures.

FTA™ Elute cards significantly improve the quantity and quality of STR loci directly amplified. Using FTA™ Elute cards 90 % (93 % concrete, 95 % jean, 88 % wood and 85 % soil) of PowerPlex® Fusion (5C) alleles were successfully amplified. In comparison, the traditional swab successfully amplified 52 % (36 % concrete, 85 % jean, 76 % wood and 14 % soil) Fusion alleles. Moreover, directly genotyping samples using FTA™ Elute cards significantly increased the RFU peak heights and peak height ratios of STR profiles when compared to samples genotyped using the swab.

Developing a collection method that could reduce the effect of inhibitory factors and provide a reliable measure of DNA collected would reduce the number of repeat samples minimizing the amount of time and money saved. The study demonstrates the potential of using FTA™ Elute cards as a collection tool, showing a significant improvement in results when compared to the swab.

#### **Direct DNA Amplification, Porous Materials, FTA Elute Cards**



### B4 An Investigation of Forensic DNA Methylation Profiling as a Method of Age Estimation Using High Resolution Melting (HRM)

Rachelle A. Turiello, MS\*, Prince George's Police Department, Landover, MD 20785; Cynthia B. Zeller, PhD, Towson University, Towson, MD 21252

**Learning Overview:** After attending this presentation, attendees will understand how epigenetic modification of specified regions of DNA are correlated with chronological age in human blood samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the growing body of knowledge concerning the genome-phenome relationship between methylation and human aging to provide a more accurate rendering for forensic phenotypic prediction, enhancing phenotypic interpretations, ultimately assisting law enforcement to generate better investigative leads and identify missing persons.

Broadly, the results presented here will also more clearly define the relationship between these epigenetic modifications and the illusive and continuous subject of aging and could even present implications for areas of inquiry beyond the scope of forensic science.

Forensic DNA phenotyping investigations have revealed positive correlations between select DNA candidate loci and human appearance.<sup>3</sup> Thus far, only those association studies focused on externally visible characteristics (EVCs) involving pigmentation have been successful isolating informative regions of corresponding genetic instructions and developing predictive assays.<sup>3,4,7</sup> The construction of a molecular test for chronological age would not only contribute to the creation of a profile to serve as a biological witness, but could also inform other phenotypic variants, such as hair color, hair loss, stature, and general appearance. Numerous studies have established a linear correlation between the degree of methylation at several CpG sites within the human genome and chronological age.<sup>1,2,6, 8-10</sup> It should be noted that methylation generally is a complex phenomenon which is influenced not only by genetic instruction, but also through environmental influences.<sup>5</sup> Thus, it may be more accurate to refer to age predicted via methylation patterns as *biological age* rather than *chronological age* until methylated age data has been thoroughly explored and compared to the age of the donor.

The aim of this study is to examine the utility of methylation status as an informative property for age prediction at five candidate loci in human blood samples. Locus selection was based on a predictive model proposed by Zbieć-Piekarska et. al. in a 2015 study and includes a total of 32 CpG sites at five genetic loci, ELOVL2, C1orf132, KLF14, TRIM59, and FHL2, examined to define the utility of their methylation status as a predictor for age.<sup>9</sup> The sites were evaluated in a total of 96 human blood samples ranging from 1-day-old to 94 years of age from the University of Maryland Hospital. DNA was isolated using a total of 25µl of whole blood from each sample and extracted via the organic phenol chloroform method to ensure a high yield. Resulting extracts were bisulfite treated with the EZ DNA Methylation-Gold™ Kit, Zymo Research, according to their standard protocol, and temperature cycling was accomplished with the GeneAmp PCR System 9700, Thermo Fisher Scientific. Only those bisulfites modified DNA samples yielding a minimum of 5ng/µl of DNA were used for subsequent testing. The methylation status of samples for each locus was captured via high resolution melting (HRM) by the Rotor-Gene Q, Qiagen, and data from each locus was binned into three age categories ranging from ages 1-30, 31-60, and 61-100.

The pre-experimental hypothesis posited a linear correlation between hypermethylation with increased age at CpG sites from C1orf132, TRIM59, KLF14, and FHL2, and hypomethylation with increased age at ELOVL2. Preliminary statistical results of binned age categories indicate moderate concordance between hypermethylation and advancing chronological age within the C1orf132 and FHL2 loci, while methylation detected within the remaining three gene regions was inconsistent with the pre-experimental hypothesis. Additional statistical analysis will be completed with the Rotor-Gene Q ScreenClust HRM Software, Qiagen, to normalize and cluster the multivariate data from each locus.

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### **Methylation, Phenotyping, Aging**



### B5 Optimizing the Processing of Databasing Samples Using the Hamilton® easyPunch™ STARlet

Allie Flores, BS\*, Bode Cellmark Forensics, Lorton, VA 22079; Heather Sarik, MS, Bode Cellmark Forensics, Lorton, VA 22079; Kevin W.P. Miller, PhD, Hamilton Robotics, Reno, NV 89501; Daniel Watsula, MS, Bode Cellmark Forensics, Lorton, VA 22079; Robert A. Bever, PhD, Bode Cellmark Forensics, Lorton, VA 22079; Kristen Naughton, BS, Bode Technology, Lorton, VA 22079

**Learning Overview:** After attending this presentation, attendees will gain an increased understanding of the capabilities of a fully automated punching and liquid handling instrument for reference sample processing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing knowledge to those laboratories faced with a backlog of samples and outside pressure to decrease turnaround times.

The passing of new legislation allowing for the collection of DNA samples from arrestees has led to some laboratories facing a significant increase in the number of samples submitted. Frequently, a crime lab's databasing section's budget and staff have not increased proportionally, creating a backlog situation. Direct amplification of reference samples has increased efficiency compared to the traditional extraction, quantification, amplification method but still requires staff to either manually remove 1.2mm punches or manually feed a semi-automated instrument. Subsequently, the analyst or technician must perform the amplification setup as a separate process and procedure.

This study evaluated the Hamilton® easyPunch™ STARlet and its ability to improve the efficiency and throughput of a databasing section. The easyPunch™ is designed as an "all-in-one" system that will provide both punching and liquid handling with minimum human interaction. Theoretically, an analyst or technician would load the instrument, initialize the run, and return at the end with a fully punched and master mix added 96-well plate ready for a thermal cyclor.

The adoption of any new method or technology requires careful consideration to ensure that solving one bottleneck does not result in creation of another. Increasing the throughput on the front end of punching and amplification is only successful if the quality of the data is consistent with the previous method. This study compared both the throughput and data quality from samples processed with manual or semi-automated direct amplification methods to the fully automated method.

Three direct amplification chemistries manufactured by three different companies were evaluated as part of this study. ThermoFisher's Globalfiler™ Express, Promega's PowerPlex® Fusion 6C, and Qiagen® Investigator 24plex GO! were chosen as they all contain the CODIS core 20 loci and contain additional Y chromosome specific loci. By testing a variety of amplification kits, the resulting data is applicable and beneficial to most databasing sections.

Ninety (n=90) Bode Buccal 2 collected samples were processed with each of the amplification kits using the Hamilton easyPunch™ STARlet as well as manually punching and adding the amplification reaction mix. The resulting DNA profiles were analyzed using appropriate laboratory analytical and stochastic thresholds. The data metrics recorded and compared included first pass success rate, average locus peak height, and average intra-color balance. Additional quality control metrics evaluating positive and negative controls as well as sample integrity were completed.

The optimized protocol provided a method to obtain a 96-well plate containing both lysed sample punches and amplification master mix in under two hours with minimal human interaction. The plate is then sealed and centrifuged off deck prior to continuing with downstream processing. All resulting positive and negative control samples provided results consistent with manual amplification set up. Zero to two clean punches were evaluated to prevent sample carryover, results indicated that one clean punch optimized sample integrity and punching efficiency. All samples provided profiles that met laboratory guidelines for each kit with regards to analytical and stochastic thresholds. The results from this study show that a fully automated platform can increase a laboratory's efficiency without decreasing profile quality or success rates.

#### Direct Amplification, Databasing, Efficiency



### B6 Evaluating *In Vitro* Seminal Fluid and Saliva Biomarker Degradation Using a Mass Spectrometry-Based Serological Assay

Catherine O. Brown, MSFS\*, Center for Forensic Science Research & Education, Willow Grove, PA 19090; Janelle Leo, BS, Philadelphia, PA 19150; Phillip Danielson, PhD, University of Denver, Denver, CO 80210; Kevin M. Legg, PhD, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Heather E. McKiernan, MSFS, Center for Forensic Science Research & Education, Willow Grove, PA 19090

**Learning Overview:** After attending this presentation, attendees will understand the effects of endogenous degradation on the selection of protein biomarkers for seminal fluid and saliva using emerging body fluid identification methodologies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by evaluating the stability of peptide biomarkers for serological testing of sexual assault evidence using protein mass spectrometry.

The use of protein mass spectrometry for serological testing of sexual assault evidence has demonstrated increased specificity and sensitivity over current antibody-based techniques. However, loss of sample due to natural removal of target material through vaginal drainage as well as breakdown of biomarkers by endogenous protease enzymes within the vaginal vault make the detection of seminal fluid and saliva in sexual assault samples more challenging.

Protein denaturation via naturally occurring protease activity is one limitation affecting post-coital detection intervals for immunochromatographic assays. These testing devices rely on the intact conformational shape of target proteins for antibody binding and detection. However, proteomic mass spectrometry-based approaches do not require conformation protein integrity as samples are subject to enzymatic digestion prior to analysis. It is important to understand whether proteolytic digestion of seminal fluid and saliva targets in the vaginal vault occurs within a target peptide sequence. This would provide additional information regarding biomarker stability ensuring accurate detection and identification in low level and degraded sample types (i.e., extended post-coital interval sexual assault samples).

Three seminal fluid biomarkers (Semenogelin 1, Semenogelin 2, Prostate Specific Antigen) and two saliva biomarkers (Alpha-Amylase, Cystatin-SA) were selected for evaluation. To eliminate the effects of natural drainage on target material and isolate the factor of interest, endogenous proteolytic degradation in the vaginal vault, *in vitro* incubations were performed. Seminal fluid- and saliva-free vaginal swabs were acquired from 10 female volunteers and extracted and pooled. Seminal fluid and saliva were obtained from a single male donor. Samples were prepared in triplicate by diluting neat seminal fluid 1:100 and neat saliva 1:50 with liquid vaginal extract. Seminal fluid samples were incubated at 37° C for 1, 2, 3, 6, 8, 10, and 12 days and saliva samples were incubated at 37° C for 1, 2, 3, 4, and 5 days. An immediate collection at Day 0 served as a positive control while blank vaginal extract served as a negative control. Samples underwent a tryptic protein digestion and C18 SPE clean-up on an Agilent® AssayMAP Bravo automation platform prior to analysis. An Agilent® 6495 triple quadrupole mass spectrometer coupled to a 1290 series liquid chromatograph was utilized for this study with an Agilent® ZORBAX 300 SB-C18 column. Separation was performed over 10 minutes with a 90% acetonitrile gradient.

Seminal fluid and saliva biomarkers were quantified using synthetic peptide standards (0.5 fmol/μL to 1 pmol/μL). Isotopically-labeled internal positive controls were used to evaluate sample response. For peptide LSEP (PSA), the initial concentration was 90 fmol/μL. Concentrations decreased in a linear manner, with calculated concentrations of 61 fmol/μL, 27 fmol/μL, and 6 fmol/μL at Days 1, 2, and 3 respectively. LSEP was not detected after three days of incubation. The QITI peptide (Sg 1) held an initial concentration of 2100 fmol/μL over three days of incubation, then began to decrease (1265 fmol/μL Day 6 and 488 fmol/μL Day 8). This peptide marker was not detected after eight days of incubation. Semenogelin 2 peptide GSIS exhibited a similar pattern to QITI. Peptide LSGL (Alpha-Amylase) had an initial concentration of 9 fmol/μL, decreasing to 5 fmol/μL at Day 1, and 3.5 fmol/μL at Day 2 where it remained for Days 3 to 5. Cystatin-SA peptide ALHF had an initial concentration of 2.5 fmol/μL which remained stable across the 5-day study.

In conclusion, endogenous breakdown of target material was evident for PSA and Alpha-Amylase peptide targets. Semenogelin 1 and 2 peptide targets appear stable for 3 days into the post-coital interval and are detectable at quantitative values 8 days into the post-coital interval. Cystatin-SA peptide targets demonstrated stability over the full 5 days of evaluation. The selected peptide amino acid sequence of target protein biomarkers should be considered, not only for specificity purposes, but to ensure the endogenous breakdown and cleavage of protein material does not negatively affect biomarker detection.

#### Forensic Science, Proteomics, Serology



### **B7 Improving the “Front End” of DNA Analysis and Trace Evidence Collection Through a Versatile Vacuum-Based Device**

*Julian L. Mendel, PhD\*, Miramar, FL 33029; DeEtta Mills, PhD, Florida International University, Miami, FL 33199*

**Learning Overview:** After attending this presentation, attendees will understand: (1) the current status of sample collection at crime scenes and in the lab; (2) the challenges that exist; (3) the potential improvements in sample collection; and (4) the development of a novel device created for this purpose.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the improvement of the “front end” of evidence/sample collection and by providing an alternative tool that could significantly improve our ability to obtain the most information from often cryptic evidence commonly encountered at crime scenes.

Much of the evidence found at crime scenes exist in trace amounts. Low template (LT) DNA, chemical residue, small particulates and fibers are all commonly encountered trace evidence that can be critical to forensic investigations. With respect to DNA analysis the vast majority of research has focused on improving the downstream analysis steps, whether through increasing the sensitivity of PCR or by developing interpretation guidelines for LT DNA profiles. While advances have been made in these areas of the analysis workflow, much of the stochastic errors and amplification artifacts still remain a problem. In contrast, little work has been done on enhancing the “front end” of the DNA analysis workflow such as sample collection. Currently sample collection of touch DNA, particulates, liquids and fibers, involves either swabbing or tape lifts. Studies have demonstrated the shortcomings of these methods especially with respect to DNA collection with swabs. Much of the DNA collected remains trapped within the fibers of the swab during extraction which makes downstream analysis of already low amounts of sample more difficult.

The goal of this study was to develop a novel collection method that would improve the amount of sample recovered and released during extraction which would in turn eliminate many of the problems encountered downstream. To this end a Venturiq based vacuum device (VVD) (Patent Pending US20170212015A1) was designed and implemented. The VVD is field portable, and through the use of various attachments can handle a variety of sample collection tasks in both wet and dry modes. The current study completed three trials for Initial evaluation of the VVD.

*Trial 1:* 100 individual touch samples were obtained through the manipulation of sterilized stainless-steel bars and the resulting samples collected with the VVD were subjected to DNA extraction and quantification using real-time PCR. *Trial 2:* A known number of microscopic pollen grains were applied in solution to cotton fabric and allowed to dry followed by collection using the VVD and repeated two more times. The collected pollen grains were then counted to determine the collection efficiency. *Trial 3:* To demonstrate the ability to collect sample from within a large object in the field the device was applied to collect a fungal pathogen growing within the interior of a living tree, the resulting sample was then plated onto agar plates to confirm growth of the fungi. The results from trial 1 demonstrated an average of 2.16 ng ( $\pm 4.94$  ng) of genomic DNA and  $1.82 \times 10^5$  total copy number ( $\pm 4.02 \times 10^5$ ) of mtDNA were recovered for potential profiling and analyses. Trial 2 resulted in 70% ( $\pm 9.4$  %) of the pollen being recovered. Lastly trial 3 resulted in the successful growth of fungal spores collected from the interior of the tree on malt extract agar plates.

The results of the three trials demonstrate the ability of the VVD to collect a variety of sample types successfully. Further evaluations are underway to optimize the device and evaluate its ability to collect additional forensically relevant samples from various substrate and surface types including rough surfaces.

#### **Sample Collection, Vacuum, DNA**





### B8 The Development of a Polymerase Chain Reaction (PCR) High-Resolution Melt (HRM) Assay for Wormwood (*Artemisia Absinthium*)

Brianna D. Kiesel, BA\*, Towson University - Chemistry Department, Towson, MD 21252; Kelly M. Elkins, PhD\*, Towson University, Towson, MD 21252

**Learning Overview:** After attending this presentation, attendees will understand how to develop DNA primers to detect legal high species including artemisia absinthium (wormwood) and the identification of this and other legal high species using a real-time polymerase chain reaction (PCR) high resolution melt (HRM) assay.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by expanding on existing assays that detect legal highs. The focus will be on the development of primers for artemisia absinthium and multiplexing the results with other legal high species to provide a fast and cost-effective way to detect for plant material.

The medicinal properties of wormwood have been celebrated for thousands of years mainly as a way to rid the body of intestinal ringworms but have also been used to treat malaria and repel bugs. During the 18<sup>th</sup> and 19<sup>th</sup> Centuries, wormwood was distilled to create the spirit absinthe. Wormwood contains alpha-thujone and beta-thujone, which can cause hallucinations when consumed in concentrated amounts.<sup>1,2</sup> Although there are herbal medicinal properties of wormwood, doctors and researchers noticed that there was an increase in neurological disorders and epileptic-like episodes that users experienced. Originally believed to stimulate cannabinoid receptors, research has since determined that a high concentration of thujone can block the gamma-aminobutyric acid (GABA) receptors in the brain. Several countries enacted a ban on the sale of absinthe, including the United States in the early 20<sup>th</sup> Century. In 2007, the United States lifted the ban on absinthe, which has quickly become popular in the bar scene, even prompting a National Absinthe Day. In 2018, *The Baltimore Sun* reported that a local bar has a Happy Hour dedicated to discounted absinthe once a week.<sup>3</sup>

Even though the sale of absinthe is legal, the Food and Drug Administration monitors the levels of thujone, and limits 10 parts per million of thujone in absinthe. Although thujone is monitored by the Food and Drug Administration, the Drug Enforcement Agency's Controlled Substances Act does not regulate materials that contain the compound. Thujone extracted from wormwood can be ingested in a tea, oil, or smoked as a pellet to experience a "legal high." Obtaining wormwood seeds are legal and can easily be purchased online and in stores throughout the United States.

DNA primers were developed based on the genomic information available, and the assay used real-time polymerase chain reaction high resolution melt (PCR HRM). Radiant Green was used to detect trace biological material from *A. absinthium* and evaluate primer specificity; the melt temperature of the primers was  $84.75 \pm 0.068$  °C. A serial dilution of *A. absinthium* proved that the assay was sensitive to DNA as low as 0.01 ng, and the size of the amplicon was estimated using an agarose gel. The specificity of the assay developed, as well as the results of duplexing this assay against another plant used to obtain a legal high, *Datura stramonium* (jimson weed). In a duplex using LC Green Plus dye, the *A. absinthium* melt temperature is 87.96 °C and the *D. stramonium* melt temperature is 76.20 °C.

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#### Wormwood, Real-Time PCR, High Resolution Melt



## B9 An Improved Swabbing Method for the Collection of DNA From Fired Cartridge Cases

Glenn P. Fahrig\*, Bureau of ATFE, Beltsville, MD 20705; Gregory A. Peiffer, PhD, Bureau of ATFE, Beltsville, MD 20705-1250; Todd W. Bille, MS, Bureau of ATFE, Ammendale, MD 20705

**Learning Overview:** After this presentation, attendees will be informed of the methods evaluated at the Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF) Laboratory to improve DNA recovery from fired cartridge cases. This presentation suggests a variation on the traditional double swab method to increase DNA recovery from brass/copper cartridge cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the potential DNA evidence on fired cartridge cases and provide justification for a new collection method that can be implemented into an existing workflow without interfering with downstream examination by other disciplines (such as tool mark examination).

Items that have been handled or manipulated, such as cartridge cases, can provide a source of touch DNA evidence that can be critical to forensic investigations. Generation of informative DNA profiles from fired cartridge cases, particularly those containing copper/brass, can be problematic due to the low level of DNA deposition, degradation introduced during the act of firing, and/or the potential for oxidative damage to nucleic acids from the substrate.<sup>1</sup> Methods designed to maximize the recovery of high-quality DNA from these evidence types may increase the success of obtaining probative DNA information. To this end, several aspects of the DNA collection process were investigated for their contribution to improved recovery and preservation of DNA quality from fired cartridge cases in this study. DNA yield was addressed by evaluating multiple swab types; oxidative damage was addressed by incorporating copper-binding additives into the swabbing solution. Additionally, the interval between deposition and DNA collection was reduced to optimize the DNA recovery. Brass 9mm cartridges were handled by volunteers for 30 seconds and loaded into an ammunition magazine, fired at a testing range, and processed for DNA collection. Samples were collected utilizing the double swab technique (wet/dry) with either sterile water or with a swabbing solution that contained bovine serum albumin (BSA) and a copper-binding tripeptide (glycyl glycyl histidine). DNA recovery was also compared when DNA collection was performed using foam popule swabs instead of cotton swabs. After DNA collection with the described methods, all samples were processed through extraction, quantitation, amplification, and typing according to ATF standard operating procedures. Quantitation results and profile peak heights/quality were assessed and compared between methods.

Use of a BSA/tripeptide swabbing solution resulted in an increase in both DNA recovery and quality as evidenced by both quantitation and electropherogram data. However, when the tripeptide solution was spiked directly into an extraction preparation to evaluate possible effects on downstream processes, DNA yield was significantly reduced, indicating a potential negative effect of the tripeptide that requires further study. The foam popule swabs recovered more DNA from the cartridge cases than traditional cotton swabs. However, foreign background DNA was detected on a subset of new unused foam popule swabs. Initial investigation indicates that a reduction in the time intervals between deposition, collection, and extraction may be another potential contributing factor to DNA recovery success observed across all methods tested thus far and will be further evaluated. Overall, the results in this study show that when the BSA/tripeptide swabbing solution was used in conjunction with foam popule swabs, 6/11 (>50%) of the fired cartridge cases yielded more than 75% of the alleles of the individual that loaded the ammunition magazine and 8/11 (>70%) of the fired cartridge cases yielded more than 50% of the loader alleles.

Fired cartridge case evidence is a valuable source of informative DNA profiles and should not be viewed as a “last resort” or a “lost cause” by scientific investigators. As more powerful methods become available, forensic laboratories can and should be positioned to accept fired cartridge case evidence routinely in a manner that is not overly burdensome on existing workflow and provides pertinent information to the criminal justice community.<sup>2-5</sup>

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### Touch DNA, Cartridge Cases, DNA Recovery



### B10 Quadrat Sampling of Splashing Incident Evidence for Urine Stain Identification

*Shelby Carlson\*, Lombard, IL 60148*

**Learning Overview:** The goal of this presentation is to describe a sampling method for urine stain identification based on the ecological technique of quadrat sampling. Attendees will learn of a strategy for sampling and testing dark fabrics on which fluorescence of urine under common alternate light sources may not be observable.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a testing framework for laboratories supporting corrections or other institutions for which urine identification from dark fabrics is required.

Described is an evidence sampling method devised for identifying urine on dark-colored fabrics based on the classic ecological technique of quadrat sampling. Upon award of a contract for forensic body fluid identification on 400+ correctional officer uniforms annually, an approach was required for urine identification in alleged “splashing” incidents. Urine applied to sample agency uniform shirts and pants, primarily midnight navy and/or black and of various fabric compositions, was not visible under ambient light and did not produce observable fluorescence using any alternative light sources tested. Therefore, an alternative sampling method was required and devised for urine identification testing on evidence items with no observable staining.

Both water and a mild detergent were evaluated as wetting solutions for swabbing large areas of fabric evidence. For either collection solution, wetting the substrate with the fluid followed by dry swabbing produced stronger signal on RSID™-Urine than swabbing dry fabric with a wetted swab. Fabric cuttings were demonstrated to produce more frequent and stronger positive results from control stains on a variety of fabric types than any swabbing technique. However, the size of each cutting was limited to 150 mm<sup>2</sup> for the desired extraction volume of 200 µL. A final, “quadrat-based” procedure reminiscent of strategies used in ecological surveying was adopted: (1) based on the event description, a region of interest (ROI) is identified; (2) within every 150 cm<sup>2</sup> area, two 150 mm<sup>2</sup> cuttings are taken at random, thus directly sampling ~2% of the ROI; (3) the remaining 98% of the ROI is sampled by wetting the fabric with a 1% Triton-X solution and swabbing up to three 150 cm<sup>2</sup> areas per swab; and (4) a cutting and swab of fabric outside the ROI are taken as substrate controls from each item.

Method validation demonstrated that quadrat-based sampling could produce RSID™-Urine positive results from uniforms splashed with human urine without visible or fluorescent stains. Casework urine testing has to date produced positive results from 13.5% of items with visible staining and 9.4% of cases tested using quadrat-based sampling of unstained items.

#### Urine, Sampling, Quadrat



### B11 A Comparative Study of Differential DNA Extraction Methods in United States Laboratories

Jenna K. Sweet\*, Aurora, CO 80016; Kelly L. Knight, MS, GMU Forensic Science Program, Fairfax, VA 22030

**Learning Overview:** The goal of this presentation is to assess the different methods of differential DNA extraction of mock sexual assault kits employed by labs across the United States by comparing male to female DNA ratios.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by trying to change how individual labs process their rape kits and evidence.

Differential DNA extraction is the process of separating the male fraction (sperm) from the female epithelial fraction. There are numerous methods of separating these two fractions that are employed in labs throughout the country, depending on population, location, funding, and other factors

Although the prevalence of sexual assault cases has decreased in the past decade, there is still a sexual assault that occurs in the United States every 98 seconds.<sup>1</sup> If the victim chooses to go to the hospital after a sexual assault, typically a sexual assault kit (SAK) is collected from the victim (normally a female), which includes swabs from the anus, vagina, and mouth.<sup>2</sup> These swabs contain both DNA from the male perpetrator and the female victim. However, sometimes the ratio of male:female DNA can be around 1:100, depending on how long the victim takes to come forward. This can make the isolation of the male DNA fraction very difficult. It is important to obtain as much male DNA as possible, so that there is a greater chance of getting a DNA profile to identify the perpetrator.

For this project, labs across the country were asked to participate by filling out a questionnaire, describing the steps used in their extraction, and then by performing differential DNA extraction on five samples with varying male:female DNA ratios. These samples will be created in a laboratory at George Mason University using sperm from a donor and saliva and will have variable ratios such as 1:10 and 1:50. The participating laboratories will be asked to perform DNA extraction, quantification and profiling. Finally, the labs will send back the DNA profiles from each of the samples to be analyzed. This idea was a modification of similar research done in Switzerland.<sup>3</sup>

The results expected from this research include the questionnaire and the DNA profiles, which will be compared against one another. The ratios resulting quantification will be considered, as well the methods, reagents, and other factors listed on the questionnaire, hoping to determine which ones produce more favorable results. This data could then be used by the scientific community to be better equipped at testing real sexual assault kits. Hopefully, this study will impact how individual labs process their rape kits and evidence, and possibly change the way in which other labs process theirs.

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#### Differential DNA Extraction, Sexual Assault Kits, DNA Profiling

### B12 Confirming Statistical Phased Single Nucleotide Polymorphisms (SNPs) Haplotype Data of 74 Microhaplotypes (MH) Across a Global Set of Populations by Massively Parallel Sequencing (MPS)

Fabio Oldoni, PhD, The George Washington University, Washington, DC 20007; Leena Yoon\*, Tysons, VA 22102; Sathya Prakash Harihar, The George Washington University, Department of Forensic Science, Washington, DC 20007; Aishwaryaa Subramanian, The George Washington University, Washington, DC 20052; Drew A. Bader, AB, Washington, DC 20007; Sharon C. Wootton, PhD, South San Francisco, CA 94080; Robert Lagacé, BS, Thermo Fisher Scientific, South San Francisco, CA 94080; Ryo Hasegawa, BS, Foster City, CA 94404; Joseph P. Chang, BS, Thermo Fisher Scientific, South San Francisco, CA 94080; Kenneth Kidd, PhD, Yale University School of Medicine, New Haven, CT 06520; Daniele S. Podini, PhD, The George Washington University, Department of Forensic Science, Washington, DC 20007

**Learning Overview:** After attending this presentation, attendees will be able to understand the potential of using Massively Parallel Sequencing (MPS) technology for acquiring haplotype data of short DNA regions containing microhaplotypes (MHs).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of using an emerging sequencing approach to generate accurate haplotype information.

Microhaplotypes are loci of two or more single nucleotide polymorphisms (SNPs) within a short distance from each other (< 300 nucleotides) with three or more allelic combinations.<sup>1</sup> These multi-SNP loci have small amplicon size, no stutter peak, and lower mutation rate than short tandem repeats (STRs), which make them candidate markers for human identification, mixture deconvolution, and ancestry prediction.<sup>2</sup> Although different approaches including fast TaqMan<sup>®</sup> assay and conventional Sanger sequencing can be used for SNP typing, these methods do not allow determining the *cis/trans* relationship between individual SNP alleles (i.e., phase) within the same amplicon. Consequentially, statistical Bayesian-based software such as PHASE, designed to reconstruct haplotype patterns from genotype data, while accurate on a population level has the potential to make mistakes on an individual level and for rare alleles.<sup>3</sup> Also if there are other SNPs within the target region that vary, these will be undetected using such approach as it focuses only on the selected SNPs that define the locus. Conversely, high-throughput sequencing technology enables the specific cloning and sequencing of each individual DNA strand, and thus distinguishing the parental haplotypes at a given locus, while also detecting other potentially discriminating SNP variations within the entire region sequenced. For forensic implementation of MHs, allele population frequencies are critical as they vary across populations and significantly more than STRs. Here, the authors focused on the comparison of statistically phase-inferred haplotypes to MPS determined haplotypes generated from 74 MH loci genotyped on a large set of population samples.

In this study, the authors selected a worldwide population set of 539 samples representative of Africa (40 Sandawe, 38 Hausa), Europe (48 Danes, 53 Khanty), South Central Asia (119 Laotians, 30 Keralites), East Asia (54 Koreans, 42 Atayal), Native America (94 Mexican Pimas) and Oceania (21 Papua New Guineans). All population samples were genotyped using the TaqMan<sup>®</sup> assay and SNP haplotypes of 74 MH loci computationally inferred by PHASE. To determine the exact phase of parental SNP haplotypes at each MH locus, the authors genotyped all DNA samples using a newly-developed MPS multiplex panel of 74 MH markers implemented on the Ion Chef<sup>™</sup> and Ion S5<sup>™</sup> (Thermo Fisher Scientific) platform.<sup>4</sup> The MPS panel of 74 MH loci totalling 230 SNPs was specifically developed for enhancing ancestry prediction and mixture deconvolution capabilities.

As expected, PHASE provided accurate haplotype prediction at the individual level when SNPs were homozygous within a MH allele and when no more than one site was heterozygous. A few differences were identified between statistically phased haplotypes and MPS determined haplotypes in haplotypes with multiple heterozygous SNPs within an individual. These were due either to missing/mistyped SNPs by TaqMan<sup>®</sup> assay or incorrect phase estimation by PHASE.

The preliminary findings indicate that overall statistical phasing provides accurate haplotype reconstruction for population allele frequency inference, particularly for common alleles. This supports the fact that computational haplotype phasing is a valuable and inexpensive screening approach as it can use databases that are already available (e.g., ALFRED).<sup>5</sup> However, going forward these are likely to be replaced by the confirmatory MPS methodology in light of its increasing cost-effectiveness and more importantly as it allows the detection of rare haplotypes caused by variations within the target region of SNPs not originally used to define the locus.

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#### Microhaplotypes, Massively Parallel Sequencing, Phased and Sequenced Haplotypes



### **B13 A Biological Sample and Fingerprint Collection From Various Surfaces: Which Comes First?**

*Aylin Yalçın Saribey, PhD\*, Uskudar University, Istanbul, TURKEY; Tugba Ünsal, PhD, Üsküdar University Department of Forensic Science, Istanbul 34662, TURKEY; Kaan Yilancioglu, PhD, Üsküdar University, Istanbul 34662, TURKEY; Sevil Atasoy, PhD, Uskudar Universitesi Adli Bilimler Enstitüsü, Istanbul, TURKEY*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### **B14 Building a Face for a Case: Advanced Investigative Leads From Forensic DNA Phenotyping and Prediction Markers**

*Gregoreese Willocks\*, Christiansted, VI 00820; Ismail M. Sebetan, MD, PhD, National University, La Jolla, CA 92037-1011; Paul Stein, PhD, National University, La Jolla, CA 92037; Vivian L. Stafford, MFS, San Diego, CA 92138*

**Learning Overview:** After attending this presentation, attendees will be better informed regarding which forensic DNA phenotyping prediction marker tools are more reliable for developing externally visible characteristics such as skin, hair, and eye color. Attendees will also appreciate and understand the value of using these biometric predictions for investigative leads for potential suspect identification in criminal casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving knowledge and increasing the fundamental understanding regarding the prediction reliabilities determined by this research. The forensic science community will be able to use this information in conjunction with published prediction tool accuracies to ascertain which tool is better suited for the marker(s) of interest.

Suspect identification plays an important role in criminal investigations. Without a suspect, a crime cannot be solved. Law enforcement agencies depend on their selected prediction tools not only for accuracy but also for reliability. Reliability is the key to identifying potential suspects. Genetic variations in single nucleotide polymorphisms (SNPs) provide very useful phenotypic markers about the physical characteristics of an individual. The externally visible characteristics (EVCs) can identify a face of a suspect.

This presentation will discuss the reliability of Forensic DNA Phenotyping prediction tools for SNPs used to predict externally visible characteristics. The 8-Plex and 7-Plex phenotype prediction tools for skin color were analyzed for reliability. A Hirisplex prediction tool was analyzed for hair and eye color prediction reliability, and Irisplex, 8-Plex, and 7-Plex prediction tools were analyzed for eye color prediction reliability in various populations.

The results of this study indicate that: (1) suspect identification from eyewitness statements is an unreliable source of identification, with an error rate greater than 75%; (2) with an 8-Plex phenotype prediction tool, successful identification outcomes approach 81% and with the 7-Plex phenotype prediction tool, successful prediction outcome is 62%. Skin color phenotype predictions are significantly more reliable with the 8-Plex compared to the 7-Plex model ( $p$ -values  $<0.05$ ); (3) IrisPlex tool results in significantly higher eye color prediction rates than using the 7-Plex or 8-Plex tools ( $p$ -values  $<0.05$ ). All three prediction tools had greater iris color prediction failures in the European population in the green and/or blue eye color bins. IrisPlex was the only eye color prediction tool that also had a significant number of failures in the green eye color prediction bins for mixed populations; (4) there was no statistically significant difference between HirisPlex reliabilities for hair and eye color predictions ( $p$ -values  $>0.05$ ). HirisPlex eye color predictions resulted in an intrinsically similar pattern using the IrisPlex, 7-Plex, and 8-Plex tools, where the highest number of failures were in the intermediate eye color category; and (5) DNA-based “witnesses” created from forensic DNA phenotyping facial composites resulted in solved cases proven by this study to be successful at a significance level greater than  $>75\%$ .

This empirical research provides data that supports the reliability of forensic DNA phenotyping prediction tools. The data demonstrated that the prediction tools evaluated in this study can be utilized for generating reliable facial reconstruction composites to provide law enforcement with advanced investigative leads. Thus, when facial composites are published for suspect identification law enforcement agencies can be confident that they will lead to the right suspect.

#### **Forensic DNA Phenotyping, Single Nucleotide Polymorphisms, Externally Visible Characteristics**



## B15 A Statistical Comparison of Mass Spectral Data for Positional Isomer Differentiation

Emma L. Stuhmer, BS\*, Okemos, MI 48864; Victoria L. McGuffin, PhD, Michigan State University, East Lansing, MI 48824-1322; Ruth Waddell Smith, PhD, Michigan State University, East Lansing, MI 48824

**Learning Overview:** After attending this presentation, attendees will know about a method for the statistical comparison of mass spectral data to increase confidence in controlled substance identification, and specifically the application of the method to compare mass spectra of positional isomers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a method to compare mass spectral data to identify and differentiate positional isomers with statistical confidence.

Suspected controlled substances are typically analyzed by gas chromatography-mass spectrometry (GC/MS) and the mass spectrum is compared to that of a reference material or library database for identification purposes. However, the identification is typically based on visual comparison of the spectra. With the increased prevalence of novel psychoactive substances, including positional isomers, identification in this manner is challenging due to high structural similarity among compounds.

Previous laboratory work developed a statistical comparison method to compare two mass spectra (e.g., spectrum of the submitted sample compared to spectrum of the reference material). The two spectra are compared using a Welch's *t*-test to assess the relative intensities at every mass-to-charge ( $m/z$ ) value in the scan range. At each  $m/z$  value, the tested null hypothesis is that the relative intensities are not statistically different, whereas the alternative hypothesis is that the relative intensities are statistically different at the confidence level selected for the test. If the null hypothesis is accepted at every  $m/z$  value, the two spectra are statistically indistinguishable. In these instances, a random match probability (RMP) is calculated indicating the probability that the mass spectral fragmentation pattern occurred by random chance alone. In contrast, if the alternative hypothesis is accepted at any  $m/z$  value, the two spectra are statistically distinguishable and the ions responsible for discrimination are identified. Successful application of the method was previously demonstrated for the statistical association and discrimination of a set of structurally similar amphetamine-type stimulants.

The work presented demonstrates application of the statistical comparison method specifically for the differentiation of positional isomers. Two sets of isomers were considered: Set 1 contained 2-fluoromethamphetamine (2-FMA), 3-fluoromethamphetamine (3-FMA), and 4-fluoromethamphetamine (4-FMA); Set 2 contained 2-ethylmethcathinone (2-EMC), 3-ethylmethcathinone (3-EMC), and 4-ethylmethcathinone (4-EMC). Each set of isomers was analyzed by GC/MS using two different instruments and with different injection parameters (e.g., different split ratios). Isomers were also analyzed by GC-time of flight mass spectrometry to collect accurate mass data, which were used to determine elemental formulae of fragment ions.

Initial comparisons show promise in the ability of the method to statistically discriminate positional isomers within each set. For example, for the FMA isomers analyzed on the same instrument with a 50:1 split ratio, 2-FMA was discriminated from both 3-FMA and 4-FMA at the 99% confidence level with up to 10 ions responsible for discrimination. Ions responsible for discrimination included  $m/z$  109 [C<sub>7</sub>H<sub>6</sub>F]<sup>+</sup> and  $m/z$  117 [C<sub>8</sub>H<sub>7</sub>N]<sup>+</sup>, with differentiation based on differences in relative intensities among the isomers. Discrimination of 3-FMA from 4-FMA was possible at the 99% confidence level, this time with 5 ions responsible for discrimination. At a 100:1 split ratio, discrimination was still observed among the isomers, albeit with fewer ions due to less representative spectra.

For the EMC isomers analyzed on the same instrument, differentiation was possible at the 99.9% confidence level. For example, with a 50:1 split ratio, 2-EMC was distinguished from 3-EMC with up to 12 discriminating ions and from 4-EMC with up to 16 discriminating ions. Discriminating ions included  $m/z$  104 [C<sub>8</sub>H<sub>8</sub>]<sup>+</sup>,  $m/z$  131 [C<sub>9</sub>H<sub>9</sub>N]<sup>+</sup>, and  $m/z$  146 [C<sub>10</sub>H<sub>12</sub>N]<sup>+</sup>, with differentiation based on differences in relative intensity among the three isomers. Differentiation between 3-EMC and 4-EMC was also observed at the 99.9% confidence level, albeit with only one ion ( $m/z$  133, [C<sub>9</sub>H<sub>9</sub>O]<sup>+</sup>) responsible for discrimination.

This presentation will discuss the application of the statistical comparison method to two sets of positional isomers and describe the chemical significance of the ions responsible for discrimination. While this research focuses on positional isomers, the statistical method can be applied to compare mass spectra of any controlled substance to increase confidence in identification.

### Positional Isomers, GC/MS, Statistical Comparison





### **B16 Multiple Transfers of Drug-Contaminated Fingerprints and Their Analysis With Raman Spectroscopy**

*Victoria R. DePrimo, BS\*, Staten Island, NY 10308; Kenneth B. Zercie, MFS, New England Forensic, Madison, CT 06443; Pauline E. Leary, PhD, Smiths Detection, Stanfordville, NY 12581; Nicholas D. Petraco, PhD, John Jay College of Criminal Justice, New York, NY 10019-1007; Lisa Dadio, MS, University of New Haven, West Haven, CT 06516; Brooke W. Kammrath, PhD, University of New Haven, West Haven, CT 06516*

**Learning Overview:** After attending this presentation, attendees will understand how substrate, enhancement technique, and multiple transfers affect the detection and identification of drugs in fingerprints using Raman Spectroscopy

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by evaluating the use of Raman Spectroscopy as a sensitive and non-destructive method that can confirm the contents of an unknown illicit substance detected in a fingermark friction ridge deposit even after multiple contacts.

Trace amounts of illicit materials within fingermark friction ridge deposit have been identified from single, secondary transfers using various substrates and enhancement techniques. However, questions remain about the amount of transfers drug contaminated fingerprints can leave on different substrates. In examining how illicit materials are retained in multiple transfers of fingerprints, additional probative value can be awarded.

Raman spectroscopy has been greatly utilized in the forensic field for a wide range of sample identification including confirming the presence of substances in fingermark friction ridge deposits. Its ability to differentiate distinct features between other substances and those of a crystalline nature makes Raman a powerful tool in identification of trace amounts drug materials. Benchtop Raman Spectroscopy-identification of drug-contaminated prints is possible over multiple transfers. However, there are considerable limitations in its application to casework. Further, portable Raman Spectrometers have been useful for rapid in-situ analysis of drugs, explosives, and other materials. Although greatly beneficial for on-site analysis of these materials, limitations of small spot size and reduced sensitivity were observed when used for testing transfers of drug-contaminated prints.

This research examined the number of multiple transfers of drug contaminated fingerprints where cocaine was detected using Raman spectroscopy. Ten participants planted 20 successive drug-contaminated prints on a series of 5 different substrates (glass, tile, plastic bags, firearm casings, and firearm magazines) with specific enhancement techniques that are most commonly seen at crime scenes (cyanoacrylate, black powder, and fluorescent powder). Portable and Benchtop Raman spectrometers were employed to assess the number of successive transfers from which drug-contaminated prints can be detected from different substrates with enhancement. Results varied based on the substrate and enhancement technique. It was concluded that although enhancement with ninhydrin prohibited the detection of cocaine in any fingerprints on paper, cocaine was detected in as many as the 20th successive fingerprint after substrates were enhanced with cyanoacrylate or enhanced with black or fluorescent powder and lifted.

By understanding how these illicit materials transfer between individuals and substances handled and the associated limitations with its identification, a level of certainty can be provided in the identification of the substance present based on the spectra obtained. Associating illicit drugs with a specific fingermark places the drugs in the hands of a specific individual, aiding forensic scientists in two ways.

#### **Fingerprint Analysis, Multiple Transfers, Illicit Drugs**



### B17 The Detection of Explosive Residue on Gloves Exposed to Environmental Conditions

Shannon Lamy\*, Oro Valley, AZ 85704; Alyssa L. Marsico, PhD, University of New Haven, West Haven, CT 06516

**Learning Overview:** After attending this presentation, attendees will understand the viability of different swabbing methods in detecting explosives from gloves exposed to a range of environmental conditions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comparison of methods of recovering explosive residue from glove materials and the effect of exposure to environmental conditions.

This research will impact the forensic science community by providing a comparison of methods to recover explosive residue from glove materials and the effect of exposure to environmental conditions.

Homemade improvised explosive devices (IEDs) are commonly used across the world. It is important to identify the type of explosive used and determine the perpetrator. If gloves are worn while creating an IED, explosive residue could be present on the gloves and this can then be used as evidence to link the criminal to the device and to the explosion site. The topic of this research project is the detection of explosive residues removed from various glove materials after exposure to a range of environmental conditions. This novel research will be used to determine how well different explosive compounds can be detected from these surface types and to determine if this changes with different environmental exposure.

Specifically, the detection limits of the explosives TNT, PETN, and TATP using Gas Chromatography/Mass Spectrometry (GC/MS) retrieved from nitrile gloves, leather working gloves, cotton gloves, and mechanic gloves were investigated to determine how well each explosive can be detected on the different gloves. The explosive standards were deposited onto the gloves, then different swabbing methods were tested to determine what method is best able to extract the explosive residues from the gloves. The swabbing methods tested included rayon swabs, cotton balls, alcohol wipes, a flooding method using a solvent, and direct extraction from the glove itself. These swabbing methods were tested with two different extraction solvents; methanol and acetonitrile. The samples were then analyzed using GC/MS and the peak areas from the chromatograms were compared to determine which swabbing method was best by determining the extraction efficiency for each swabbing method. Preliminary results appear to indicate that using an alcohol wipe or a rayon swab are efficient methods to obtain TNT from cotton gloves. However, several unidentified peaks were also present in the chromatogram when using the rayon swab to extract TNT. These peaks could be trace material that was already present on the glove before the explosive was added, indicating that the rayon swab method is an extremely efficient extraction technique. However, this is still under continued investigation. Preliminary results indicate that using acetonitrile as an extraction solvent yields slightly higher recoveries of TNT than methanol.

Finally, these gloves were then exposed to environmental conditions including simulated wind, rain, shaking/movement, contamination, time, and mixing with cleaning products to determine any changes in the detection limits of the explosive compounds and to identify any changes to the compounds that may occur, including degradation or the presence of adducts. Preliminary results lead to the discovery of new unidentified peaks present in mixtures of explosive compounds with Simple Green cleaning solutions. These unidentified peaks warrant further investigation to determine if they are fragments from the cleaning product or the explosive compounds, or a cleaning agent adduct that may have formed from a reaction of the cleaner with the explosive compound when mixed.

The results of this research indicate the optimal swabbing method for different explosive compounds on a variety of gloves as well as how various conditions affect how well they are detected. These results can provide useful information in cases involving explosive residue on gloves, or similar fabric.

#### Explosive Residue, GC/MS, Environmental Conditions



**B18 WITHDRAWN**



### B19 Increased Objectivity of Shooting Distance Determinations by Spectrochemical Mapping

Courtney H. Vander Pyl\*, Department of Forensic and Investigative Science, Morgantown, WV 26506; Oriana Ovide\*, Department of Forensic and Investigative Science, Morgantown, WV 26505; Bayram Yuksel, PhD, West Virginia University, Morgantown, WV 26506-6121; Tatiana Trejos, PhD, West Virginia University, Morgantown, WV 26506

**Learning Overview:** The goal of this presentation is to show how the superior selectivity and sensitivity of Laser-Induced Breakdown Spectroscopy (LIBS) analysis, compared to color methods, will offer more reliable investigative information and more defensible results in court when a firearm is involved in a crime.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community using Laser-Induced Breakdown Spectroscopy (LIBS) to improve the scientific reliability of the detection and observation of gunshot residues on target materials.

In the most recent data from the Gun Violence Archive, there have been a total of 32,483 firearm related incidents in 2018, thus far. In the investigation of these crimes, the detection of gunshot residue (GSR) for firing distance estimations is crucial to support or reject the hypothesis of a suicide, a homicide, or an accidental shooting.

Currently, the most common method for estimating the muzzle-to-target distance is by colorimetric assays that react when lead (sodium rhodizonate test) or nitrites (Modified Griess test) are present. While these colorimetric tests are widely used in forensic laboratories, there have significant drawbacks like poor selectivity and limited scope. False positives can arise from common contaminants like dirt and oil, and dark colored or blood-stained clothing can significantly reduce the efficiency of these color analyses. As a result, there is a need to modernize and enhance the scientific validity of these examinations.

Laser-Induced Breakdown Spectroscopy (LIBS) is proposed as a more objective analytical tool that can generate spectrochemical images of the distribution of multiple GSR elements around the bullet hole. The central hypothesis of this research is that the use of LIBS will improve the scientific reliability of the detection and observation of gunshot residues on target materials. This assumption is based on the ability for LIBS to simultaneously detect multiple elements at low parts per million (ppm) levels and to have the potential to measure numerous emission lines per analyte of interest.

A calibration curve was created using 20 grey clothing samples (100% cotton) and 15 patterned clothing samples (100% cotton) shot with a 9 mm pistol at known distances (contact, 6 inches, 12 inches, 24 inches and 36 inches). These samples were used as a training set for statistical analysis. Additionally, 25 clothing samples, varying in color (grey, orange, maroon, navy, black, patterned), were shot at distances unknown to the analyst, thus blinding the test samples. A 100um laser beam was fired into the sample and radially moved 13 cm away from the entrance hole. The sample holder was automatically moved at a speed of 0.3 mm per second while acquiring a spectral signal in real time. Integration of the peaks was performed on elements of interest, including Sb (259.8 nm and 252.8nm), Pb (405.8 nm and 368.3nm), and Ba (493.4nm and 454.4nm). The elemental intensities of the signals and their relative spatial location allowed for the creation of 3D chemical images. Principal Component Analysis (PCA) and Leave-One-Out cross-validated Linear Discriminant Analysis (LDA) were used for prediction of unknown distances. As the LIBS method is practically non-destructive of the sample, the clothing items were then subjected to chemical color tests for comparison purposes.

While dark-colored or patterned backgrounds masked the soot pattern, making visual and color examinations challenging and some colored fabrics (black and navy) interfered with the Griess reagents and the amount of Pb deposited after firing some of the ammunition was below the sodium rhodizonate's limit of detection ( $1000 \pm 20$  ng), LIBS data offered improved selectivity, allowing the detection of multiple ionic and atomic emission lines per element with no interference from the fabric composition. LIBS limits of detection were superior to the rhodizonate test ( $20 \pm 4$ ng) with the added advantage of offering a more comprehensive identification of elements associated with GSR, both leaded and lead-free ammunition.

Color tests lead to misclassification of 3 out of 25 shooting distances (12%), while the LIBS method correctly classified the distance range of the unknown testing samples by Linear Discriminant Analysis.

This study is anticipated to aid in crime scene reconstruction when a firearm is involved in a criminal event. The superior selectivity and sensitivity of LIBS analysis in this application, compared to color methods, will offer more reliable investigative information and more defensible results in court.

#### LIBS, Gunshot Residue (GSR), Distance Determination



### **B20 A New Color Identification of Benzodiazepines in Blood and Pharmaceuticals: Using Cobalt Thiocyanate as Reagent**

*Zahid Mahmood, MPhil\*, Punjab Forensic Science Agency, Lahore, PUNJAB 53700, PAKISTAN*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### B21 The Application of Surface-Enhanced Raman Spectroscopy (SERS) for the Detection of Opioids

Ling Wang, MS\*, Florida International University, Miami, FL 33139

**Learning Overview:** The goal of this presentation is to describe the development and application of Surface Enhanced Raman Spectroscopy (SERS) for the presumptive determination of opioids and fentanyl. Information provided will include the optimized detection method, and the experimental and calculated spectrum.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the SERS method in the presumptive screening of opioids. The new method is fast and can rapidly distinguish opioids and fentanyl analogues.

Over the last five years, the abuse of opioids has been a critical issue to public health. The appearance of new synthetic fentanyl ever increase, the abuse of which threatens death; indeed, over 100 people in the United States die from overdose every day. Current screening methods, such as immunoassay, have difficulty detecting the full range of opioid analogues due to a wide variety of structural variations. This work shows an alternative screening method using Surface Enhanced Raman Spectroscopy (SERS) coupled with gold/silver nanostars and magnesium chloride aggregating agents. SERS is a rapid screening method that provides molecular fingerprint signals at toxicological concentrations. This work proves that this new method can distinguish fentanyl analogues and opiates at low to sub ng/mL concentrations. Additionally, this procedure is simple and fast to operate. The procedure is convenient for use in point-of-care analysis and in laboratory settings.

The SERS method utilizes the complex of gold/ silver nanostars and magnesium chloride. When the Au/Ag nanostars are mixed with magnesium chloride, these nanostars aggregate. Next drug samples are added to the aggregated nanostars and allowed to incubate 5 minutes. The solution is placed in a quartz well plate and analyzed via a Perkin Raman 400F instrument with laser excitation at 785 nm. Hot spots created between the aggregated nanostars results in localized surface plasmon field effects that produce a superior SERS enhancement. The SERS spectrum also provides a “fingerprint” that can be used to ascertain the chemical structure of target drug samples and identify individual compounds. Linear discriminant analysis and principle component analysis were also used to create a model to distinguish opioids based on the generated spectra. Additionally, in silico density functional theory modeling was applied to various fentanyl molecules and used to identify parameters necessary in calculating theoretical SERS spectra. The resultant data should prove useful in identifying unknown compounds and characterizing their interactions with the nanoparticle substrates. SERS technique has high sensitivity. Based on the aggregated nanostars method, the detectable limit of opioids can be 0.25 ng/mL to 25 ng/mL depending on the type of drug.

The SERS method permits a rapid, easily operated presumptive test for opioids. It is orthogonal to mass spectrometry and sufficiently sensitive to detect compounds at toxicological levels. As a result, it should be particularly useful for the screening of opioids and other novel psychoactive substances.

#### SERS, Opioids, Fentanyl



### **B22 The Effects of Exposure to Various Environmental Conditions on the Analytical Data of Manufactured Fibers**

*Alexis R. Weber, BS\*, Jacksonville, FL 32225; Virginia M. Maxwell, DPhil, Department of Forensic Science, West Haven, CT 06516*

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**Learning Overview:** After attending this presentation, attendees will understand how different environmental conditions affect the ability to analyze manufactured fibers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing analytical data of manufactured fibers during a time trial where the fabrics (nylon, acrylic, rayon, and polyester) were placed in different environmental conditions. The results of which inform forensic scientists of how various environments can affect the analysis of manufactured fibers.

The forensic analysis of fiber evidence involves the ability to link a questioned fiber back to its known source. Before the collection and analysis of fiber evidence, fibers are potentially exposed to various environmental conditions for an extended period of time. There has been significant research done on how natural fibers are affected by environmental conditions showing how natural fibers degrade overtime. However, there has been little research on the impact of environmental conditions on manufactured fibers. To avoid the potential of erroneous exclusions of fiber evidence, it is necessary to determine if the analytical data from manufactured fibers is measurably altered when the fabrics are exposed to environmental conditions for an extended period of time.

Colorless manufactured fabrics of nylon, polyester, acrylic, and rayon were exposed to multiple environmental conditions for a nine-month period. These conditions ranged from solid media including soil, sand, cow manure, chicken manure, mixtures of soil and manure, to liquid media including calcium chloride road pretreatment, calcium chloride road pretreatment and water, salt road pretreatment and oil. Squares of each fabric type were placed in glass containers, each stored while exposed to a different controlled environmental condition. Fibers from each fabric square were removed from the environments every two weeks and analyzed microscopically and instrumentally.

Microscopic examination of the fiber followed the standard operating procedures established by SWGMAT. The instruments used to examine the fibers after exposure were a Raman spectrometer, Fourier Transform Infrared Spectrometer (FTIR) and Ultraviolet/Visible Microspectrophotometer (UV/Vis MSP) in fluorescence mode. Comparisons of the exposed fibers were made back to the control fibers to determine if the fibers were measurably altered over time to a point where they are inconsistent with their known source. Throughout the first eight weeks of exposure, rayon completely broke down leaving no remnants behind. This provided an opportunity to compare the decomposition rate of rayon to cotton by adding cotton to the containers since rayon is a semisynthetic fiber composed of cellulose.

Microscopic analysis was used to determine if there are any significant differences in the fibers' physical appearance over time. Within the first two months of microscopic analysis there was no significant change in the microscopic properties of the manufactured fibers. Instrumental data was analyzed using statistics to determine if there were significant differences between the instrumental results over the course of the time study. With the exception of rayon, the increased strength and resilience of manufactured fibers over natural and semisynthetics should allow for there to be no significant observed changes to the analytical data from the manufactured fibers despite the exposure to various environmental conditions.

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#### **Manufactured Fibers, Environmental Conditions, Analytical Data**



### B23 The Development of a Rule-Based Protocol for Evaluating Fire Debris Using Known Ground Truth Samples and a Best Factors Algorithm

Taylor A. Wood\*, National Center of Forensic Science, Orlando, FL 32826; Mary R. Williams, MS, National Center for Forensic Science, Orlando, FL 32816-2367; Michael E. Sigman, PhD, University of Central Florida, Orlando, FL 32816

**Learning Overview:** After attending this presentation, attendees will understand how the development of a dataset of known ground truth samples can assist in developing a rule-based classification model. The use of a genetic algorithm to choose an optimal set of factors for the rule-based model will also be demonstrated.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the production of a decision tree model based on laboratory practices in fire debris analysis.

ASTM E1618-14 is the standard test method for fire debris analysis used by most fire debris analysts in the classification of neat ignitable liquids but is limited in the assistance it provides for detecting limited amounts of ignitable liquids relative to substrate pyrolysis products. Common practice is to examine total ion chromatogram (TIC) and extracted ion profiles (EIPs) for an ignitable liquid pattern, such as a distillate or gasoline pattern. However, the detection of a weak pattern attributable to ignitable liquid residue becomes difficult with substantial background from the pyrolysis product.<sup>1</sup> In this study, samples with known ground truth (i.e., the presence or absence of residues from an ignitable liquid from a specified ASTM E1618 class), were created in a known and controlled ratio of ignitable liquid to substrate. The known ground truth samples assist in the development and validation of rule-based and decision tree classification methods. The ratio of ignitable liquid to substrate was varied to provide a set of casework relevant samples. Each known ground truth sample was prepared from a single, weathered, ignitable liquid and single or multiple substrates. Known ground truth samples were classified as “IL” (containing ignitable liquid residue) or “SUB” (containing only substrate pyrolysis products). Limiting the problem to determination of two classes facilitates development of a dichotomous classification model that addresses the most forensically-significant question: does the sample contain ignitable liquid residue?

This research will outline how the method uses a genetic algorithm to choose the best factors to incorporate into the model. The best factors were chosen from a set of ASTM E1618 Table 2 ions. ASTM E1618 Table 2 lists the most common ions present in ignitable liquids and limited substrates. Many of these ions are used when constructing EIPs for the classification of ignitable liquids in the Ignitable Liquids Reference Collection Database and for identifying ignitable liquid patterns in the Substrate Database.<sup>1-3</sup> The genetic algorithm chose the best factors by minimizing the quantity 1-AUC, defined as the area under a receiver operating characteristic (ROC) curve that was generated by cross validating a decision tree model based on a select set of ions. This presentation will focus on models based upon a C 5.0 decision tree and the associated rules derived from the model. The use of the validated model will be demonstrated by evaluating large-scale burn data.<sup>4-7</sup>

*This work was supported by the National Institute of Justice, Office of Justice Programs, award 2017-IJ-CX-0023. The content of this publication does not necessarily reflect the position, or the policy of the government and no official endorsement should be inferred.*

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#### Fire Debris, Ground Truth, Rule-Based Methods





**B24 WITHDRAWN**



### **B25 The Simultaneous Detection of Rodenticides and Drugs Using Thermal Desorption Direct Analysis in Real-Time Mass Spectrometry (TD/DART®-MS)**

*Elizabeth Robinson, MS\*, National Institute of Standards and Technology, Gaithersburg, MD 20899; Edward Sisco, PhD, National Institute of Standards and Technology, Gaithersburg, MD 20899*

**Learning Overview:** After attending this presentation, attendees will better understand Thermal Desorption Direct Analysis in Real-Time Mass Spectrometry (TD-DART®-MS) for the analysis of drug evidence suspected of containing superwarfarins, such as rodenticides. Attendees will be presented with instrument optimization parameters, representative spectral analysis and sensitivities, and a discussion of the effect of competitive ionization on the identification of these compounds in complex mixtures and examples of detection of these compounds in street samples.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by providing key information for the use of TD-DART-MS for the analysis and simultaneous detection of rodenticides and drugs, such as cocaine, methamphetamine, heroin, and synthetic cannabinoids.

As drug analysis becomes increasingly complex, due to the onslaught of novel psychoactive substances (NPSs), drug chemists are being tasked to analyze more complex drug mixtures. These analyses are further complicated when additional toxic components are added to street drug samples. One class of compounds that has been reported in the past and is being encountered more frequently is rodenticides, also known as superwarfarins. These compounds, which are the active ingredient(s) in rodent poisoning, cause severe bleeding in drug users which can lead to death. In April 2018, the Centers for Disease Control (CDC) released a warning on synthetic marijuana containing these compounds and others have reported these compounds in cocaine and other drugs. More recently, the FDA also issued a warning about the health hazards of contaminated illegal synthetic cannabinoid products. While these compounds can be detected by techniques such as gas chromatography mass spectrometry (GC/MS), forensic analysts could benefit from a rapid tool to screen drug samples for superwarfarins, both for the purposes of casework and to provide information to trigger a public health response.

Direct analysis in real-time mass spectrometry (DART-MS) is an ambient ionization mass spectrometry technique that has been gaining popularity in forensic analyses due to its ease of use, minimal sample preparation, and rapid (less than 5 seconds) analysis times. DART-MS has also been shown to be able to detect a range of compounds from drugs to explosives to paints and lubricants. However, one of the concerns with traditional DART-MS analysis is the potential exposure to aerosolized sample via inhalation. This work utilizes a variant of DART-MS, thermal desorption direct analysis in real-time mass spectrometry (TD-DART-MS), which minimizes this risk by confining the region of sample introduction and the flow of subsequent aerosolized particles.

This work focused on the use of TD-DART-MS for the detection of rodenticides present in drug mixtures. The study was comprised of method development for these compounds, understanding the mass spectral response and sensitivity and also understanding the competitive ionization effects that can occur when complex mixtures of rodenticides and drugs are analyzed. A total of six rodenticides (bromadiolone, brodifacoum, chloropacinone, difenacoum, diphacinone, and pindone) were investigated. Method development included optimization and understanding the effects of the DART gas (N<sub>2</sub> vs He), the sample desorption temperature, and the Vapor flow rate as well as comparing these settings to those previously established for drug analysis. From the optimized method, representative spectral responses were collected and, as expected, the protonated molecular ion was most often found to be the predominant ion, except for Bromadiolone which had a predominant dehydroxylated molecular ion. Limits of detection were all determined to be less than 100 ng. Competitive ionization studies show minimal effects when the rodenticide is present at greater than 5 % of the mixture. Current work focusses on the ability to screen real case samples for rodenticides, providing forensic practitioners with a rapid method of screening samples that can be done safely and at the low levels required.

#### **Rodenticides, DART®-MS, Drug Analysis**



### **B26 New Psychoactive Substances (NPS): How a Forensic Institute Deals With the Lack of Certified Standards in Brazil**

*Lara R.S. Gris, MS\*, Instituto Geral de Perícias, Porto Alegre, RS 90160-093, BRAZIL; Emmanuele V. Baggio, Instituto Geral de Perícia RS, Porto Alegre 90160-093, BRAZIL; Paulini B. Wegner, Instituto Geral de Perícia RS, Porto Alegre 90160-093, BRAZIL; Patrícia D. Correa, Instituto Geral de Perícias, Porto Alegre 90160-093, BRAZIL*

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**THIS ABSTRACT WAS NOT PRESENTED.**

**B27 Portable Quadrupole-Based Gas Chromatography/Mass Spectrometer (GC/MS) Versus a GC/MS Benchtop System: An Analytical Comparison**

Tais R. Fiorentin, PhD\*, Willow Grove, PA 19090; Shana M. Hogg, PhD, Center for Forensic Science Research and Education, Willow Grove, PA 19090; David M. Martin, PhD, Drug Enforcement Administration, Educational Found, Washington, DC 20006; Thom Browne, Jr., Rubicon Global Enterprises, Huachuca City, AZ 85616; Barry K. Logan, PhD, NMS Labs/CFSRE, Willow Grove, PA 19090

**Learning Overview:** After attending this presentation, attendees will understand the efficiency of a platform newly applied to the analysis of drugs of abuse and adulterants in seized material, the FLIR G510, a portable quadrupole-based gas chromatography/mass spectrometer, as compared to a regular benchtop gas chromatography/mass spectrometry (GC/MS) system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about the accuracy and reliability of new methodologies used for on-site seized drug material screening.

Cutting agents, classified as diluents (pharmacologically inactive and readily available substances; e.g., sugars) and adulterants (pharmacologically active, more expensive and less available; e.g., phenacetin) are commonly used to increase profits and they are constantly changing over time, increasing the risks to the user’s health caused by the compounds interactions. Knowledge about cutting agents is commonly neglected either because they are not detected or not reported. This leads to a lack of information that could be useful for management of acute intoxications in hospitals or criminal investigations, or in helping in the identification of routes of trafficking.

Seventy-five mock samples were prepared by an independent researcher at different concentrations and proportions of cocaine, heroin, methamphetamine, and adulterants. Samples were screened using FLIR G510 and confirmed by Agilent GCMS. FLIR G510 was operated on full scan acquisition ranged from 43 – 425 *m/z*. 1µL sample injection was introduced into the injection port at 250°C (splitless mode). Chromatographic separation was achieved using a low thermal mass DB-5 column bundle. The oven temperature ramped from 50°C to 300°C with a heating rate of 30°C/min and a final hold at 300°C for 4 min resulting in a 13.2 min chromatographic run. GCMS analysis were conducted using a Gas chromatograph model 6890N, coupled with a mass selective detector model 5975B operated in electron impact mode, using full scan acquisition in the range 40 – 550 *m/z*. Chromatographic separation was achieved using a DB-1 column. The chromatographic conditions were as follows: injection volume of 1 µL, splitless mode, injection and detection temperature of 265°C and 300°C, respectively. The oven program temperature was: 50°C to 340°C, heating rate of 30°C/min, and hold at 340°C for 2.33 min, resulting in a 12 min chromatographic run. FLIR G510 and GCMS qualitative results were compared to assess the reliability for detecting the presence of drugs. Analysis was performed using Receiver Operating Characteristic (ROC) analysis. True positive (TP) samples were screened and confirmed positive; true negative (TN) samples were negative in both screening and confirmatory assays; false positive (FP) samples were screened positive, but the target drug was not present at the confirmatory assay; and false negative (FN) samples screened negative but subsequently tested positive for the target drug by GCMS. Sensitivity, specificity, PPV (positive predictive value), NPV (negative predictive value) and accuracy, as well as the number of TP, TN, FP and FN samples analyzed by FLIR G510 and compared with GCMS results are shown in **Table 1** for all compounds.

**Table1.** Results in percentage of the FLIR G510 evaluation in comparison with GCMS for cocaine (COC), levamisole (LEV), benzocaine (BZC), theophylline (THP), phenacetin (PHN), hydroxyzine (HYDZ), diltiazem (DTZ), acetaminophen (ACT), caffeine (CAF), heroin (HER), fentanyl (FEN), strychnine (STYC), codeine (COD), morphine (MOR), thebaine (THB), methamphetamine (METH) and ephedrine (EPH).

FLIR G510 vs. GCMS (%)									
Drug	TP	FN	FP	TN	Sensitivity	Specificity	Accuracy	PPV	NPV
COC	25	-	-	50	100	100	100	100	100
LEV	-	2	-	73	-	100	97.3	-	97.3
BZC	9	-	-	66	100	100	100	100	100
THP	4	3	-	68	57.1	100	96	100	95.7
PHN	5	-	-	70	100	100	100	100	100
HYDZ	2	3	-	70	100	100	96	100	95.8
DTZ	1	3	-	71	25	100	96	100	95.9
ACT	10	1	-	64	90.9	100	98.6	100	98.4
CAF	19	-	-	56	100	100	100	100	100



HER	21	4	-	50	84	100	94.6	100	92.5
FEN	5	-	-	70	100	100	100	100	100
STYC	3	-	-	72	100	100	100	100	100
COD	8	3	-	64	72.7	100	96	100	95.5
MOR	3	-	3	69	100	95.8	96	50	100
THB	1	1	-	73	50	100	98.6	100	98.6
METH	24	1	-	50	96	100	98.6	100	98
EPH	6	-	-	69	100	100	100	100	100

The accuracy of a test refers to the degree of agreement between the screening test results and confirmatory test results. Accuracy values close to 100% are ideal, but in some situations a higher sensitivity or specificity are preferable. Sensitivity is directly affected by the number of FN as we can see on **Table 1**. THP, DTZ and THB sensitivities are low due to the proportion of FN detected for these substances. The PPV (which evaluate the confirmability of positive value) of MOR is low in comparison with the rest of the substances due to the FP cases detected for this specific substance. In general, accuracy values were at or close to 100% classifying the FLIR G510 as a suitable tool for field-based screening in seized material analysis.

### Portable GC/MS, GC/MS, Seized Drugs



**B28 WITHDRAWN**



### **B29 Bomb Biometrics: Detection and Imaging of Markers Indicative of Explosive Compounds in Fingerprints**

*Cameron M. Longo\*, University at Albany-SUNY, Albany, NY 12222; Rabi A. Musah, PhD, State University of New York at Albany, Albany, NY 12222*

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**Learning Overview:** After attending this presentation, attendees will gain insight into how mass spectrometric techniques can be used to identify contact with explosive materials based on fingerprints or fingerprint residues, as well as how mass spectrometry imaging can be used to connect this information to an individual using specific biometric data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the potential value of evaluating the chemical content of fingerprints in addition to the physical pattern, specifically by highlighting an approach to identifying and correlating the handling of illicit or otherwise forensically-relevant compounds to an individual by using mass spectrometric techniques.

Fingerprints have been a common staple of the crime scene investigative processes for decades, and while they are extremely practical in establishing a connection to a specific person, a large portion of the available information present in a fingerprint is currently unutilized. Indeed, the chemical content of a fingerprint may hold a bounty of useful information pertinent to an investigation. This may include biomarkers indicative of a suspect's age and/or sex, medications or illicit drugs that they have ingested, or contact with other molecules. The use of a rapid, ambient ionization mass spectrometry technique would allow for fast screening that could be used to quickly flag molecules of forensic interest, while a robust imaging technique could reveal the spatial distribution of said markers and be used to link this evidence to an individual based on their unique fingerprint pattern. The work shown will demonstrate how direct analysis in real time mass spectrometry (DART-MS) can be used to quickly identify potential contact with explosive materials, while matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) can be used to map the spatial distribution of relevant markers and tie them to an individual with biometric information.

As proof of concept, dilute solutions of some common explosives or explosive precursors, such as TNT, tetryl, and nitroglycerin, were handled by subjects prior to deposition. Fingerprint residues were collected by rolling glass beads between the fingertips. The beads were submersed in solvent and sonicated briefly, after which the solvent was removed under vacuum to produce a concentrated residue. This residue was analyzed by using a JEOL AccuTOF mass spectrometer coupled with a DART-SVP ion source under conditions compatible with the compound being analyzed. Utilizing this method, peaks corresponding to either protonated whole molecules or fragments of molecules can be detected.

Fingerprints were deposited onto ITO-coated conductive glass slides, and a matrix solution of either CHCA or 9AA was applied. The sample was analyzed in two-dimensions using a JEOL S3000 SpiralTOF MALDI mass spectrometer in either positive or negative spiral mode at a spatial resolution typically between 70-80  $\mu\text{m}$ . Using this method, peaks corresponding to the explosive material can be detected. In addition, 2D ion renderings can be generated for each peak, displaying the spatial distribution of each ion detected. These are identical to the spatial distribution of endogenous molecules such as fatty acids, which aids in demonstrating the direct link between the chemical information and the donor of the fingerprint.

The chemical information stored within a fingerprint could be of great value to investigators, though it generally remains underutilized. The mass spectrometric techniques shown here can probe this content to provide additional information in investigations of crimes. DART provides this in a rapid way and can be used as a potential screening method, while MALDI is able to link this chemical information definitively to an individual using biometric data and comparisons to the patterns established by endogenous compounds.

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#### **Explosives, Mass Spectrometry, Fingerprints**



### **B30 Gunshot Residue Detection on Wounds in Stagnant Water: Scanning Electron Microscopy With Energy-Dispersive X-Ray Spectroscopy (SEM/EDX) or Inductively Coupled Plasma/Mass Spectrometry (ICP/MS)? A Preliminary Study**

*Romain Montoriol, MD\*, Service de Médecine Légale, CHU Rangueil, Toulouse, FRANCE; Céline Guilbeau Frugier, PhD, Service De Médecine Légale, Hôpital De Rangueil, Toulouse 31059, FRANCE; Sébastien Aries, PhD, Antellis, Toulouse 31400, FRANCE; Lauriane Onfroy, PhD, Antellis, Toulouse, Midi-pyrénées 31400, FRANCE; Bruno Payré, PhD, CMEAB, Faculté de Rangueil, Toulouse 31062, FRANCE; Valentin Lombardi, Laboratoire AMIS, Toulouse 31000, FRANCE; Norbert Telmon, PhD, MD, Service Medico-Judiciare, Toulouse F-31054, FRANCE; Frederic Savall, Service De Médecine Légale, Toulouse Cedex 9 31059, FRANCE*

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**Learning Overview:** After attending this presentation, attendees will understand: (1) gunshot residue detection on wounds is challenging in degraded bodies (animal model) in stagnant water with a scanning electron microscopy (SEM) coupled with EDX (energy dispersive X-ray) analysis; and (2) Inductively Coupled Plasma Mass Spectrometry (ICP-MS) might be a solution.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the use of a highly sensitive technique ICP-MS for gunshot residue detection on wounds in degraded bodies.

**Introduction:** The study of firearm projectile wounds can be of great importance in forensic practice. While the identification of firearm projectile wounds on putrefactive and degraded bodies might be challenging, the identification of entry wound and exit wounds are more difficult.

Scanning electron microscopy (SEM) coupled with Energy dispersive X-ray (EDX) analysis is the reference technique for gunshot residue (GSR) detection. While some papers have described its use on biological samples, analytical results were only qualitative. Additionally, some technical problems from background noise caused by elements in tissue interfere with element identification using SEM EDX. By contrast, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is an alternative technique for element detection with high sensitivity that has potential to be considered as a gold standard.

The objective of this preliminary study was to detect GSR using SEM-EDX and ICP-MS on gunshot wound after stagnant water immersion of the anatomical structure and to assess the possibility of entrance and exit differentiation of wounds.

**Material and Method:** Twenty-two shots were performed on animal model (sheep) by the Laboratoire de la Police Scientifique de Toulouse (technical and scientific police) at distance of 20 centimeters using a .22 long rifle (LR) manual rifle and Remington standard velocity. One thigh was used as reference (D0) and analyzed after shooting while the three others were placed in a barrel filled with stagnant water. Samples from one thigh were collected for analysis after 3, 6, and 14 days of immersion. For the analysis by SEM-EDX, each orifice was split in half with a sterile scalpel so that GSR was directly analyzed on the soft tissues with SEM-EDX and with swab for ICP-MS.

**Results:** SEM-EDX analysis of entrance wounds, showed particles were numerous compared to exit wounds. However, only Lead (Pb) was detected even at D0. Moreover, Antimony (Sb) peaks were difficult to separate from Calcium peaks. In consequence, the authors were not allowed to attest that particles detected were GSR. Immersion caused an enrichment of particles with Silicon peaks (Si). Pb was still detected but with difficulty because of silt contamination.

ICP-MS analysis showed that Pb and Sb were detected on entrance and exit wounds allowing the conclusion of possible GSR presence. Detection was not affected by water immersion. The difference in quantity of Pb and Sb particles between entrance and exit wounds allowed for distinction with a factor calculated around 14 for Pb and around 5 for Sb. Barium (Ba) quantities were low without any difference between entrance and exit wounds. However, stagnant water contained Ba.

**Discussion:** Surprisingly, SEM-EDX was not able to detect GSR properly due to washing out effect and was affected by contamination by silt.

In contrast, ICP-MS showed promising results with detection of Lead and Antimony in relatively high concentration in entry wounds with difference between entrance and exit wounds. Contamination by stagnant water silt did not impact Pb and Sb couple detection by ICP-MS. Therefore, ICP-MS is an interesting alternative technique for GSR detection when SEM-EDX failed to detect it.

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#### **Gunshot Residue, SEM/EDX, ICP/MS**





### B31 The Decomposition Clock: A Characterization of Insect Volatile Odor Profiles

*Katie E. Blonar, BS\*, Lubbock, TX 79423; Paola A. Prada, PhD, Texas Tech University, Lubbock, TX 79416*

**Learning Overview:** After attending this presentation, attendees will gain a better understanding of the volatile organic compounds emitted from collected maggots feeding from a decomposing pig cadaver and how the volatile odor pattern changes during the decomposition stages.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by providing strong scientific perspectives on insect odor profiling, by implementing a chemical study regarding odor compound identification both qualitative and semi-quantitatively throughout an animal model decomposition process. Insects are observed at nearly every death scene and are among the first creatures to arrive to a corpse. Therefore, this study will further enhance the use of insects as evidence at death scenes by providing vital information that may not be available elsewhere. The optimal implementation of insects as a source for odor biomarkers impacts the forensic field by providing a valuable, highly advantageous technique that can be used in multiple situations.

**Significance:** The benefit of this study is enhanced knowledge in the realm of optimal odor profiling of maggots from the different stages of decomposition. This research will fundamentally bridge the gap in knowledge regarding the odor profiles emitted by maggots at various decomposition stages and how this profile impacts criminal investigations, which may be lacking evidence, and provide it with a new upcoming technique.

Forensic Entomology is a growing discipline with very little scientific research regarding insects found on a dead body and how they can provide vital information that may not be found anywhere else. Perhaps due to the limited research that has been done, there are also few experts in this discipline who look at odor profiles in connection with the insects that appear. Emerging research includes looking at odor profiles of different animal cadavers as well as the odor emitted from beetles placed on a corpse. However, there are no studies that are specifically looking at the odor emitted by maggots feeding on a cadaver. The purpose of this study is to investigate the volatile odor profile within maggots found on decaying piglet cadavers as a function of decomposition stage and compare with previous literature the results obtained to see how an insect's volatile odor profile compares to human/animal decomposition models. With this information from the maggots, the utilization of insects as a sample matrix can potentially be implemented by forensic scientists using volatile biomarkers as indicators of postmortem intervals. Knowing the volatile organic compounds emitted from the cadaver insect populations will also give cadaver canine teams more information to improve training procedures in hopes of enhancing and standardizing those procedures. This study used thirteen piglet cadavers: three for a pilot study, five for a summer sampling period, and five for a fall sampling period. Instrumental evaluation utilized Divinylbenzene/Carbon/Polydimethylsiloxane (DVB/CAR/PDMS) coated Solid Phase-Microextraction (SPME) fibers, samples from which were injected into a Gas Chromatography-Mass Spectrometry (GC/MS) system for the identification of extracted volatile odor profiles of maggots at the different stages of decomposition. The pilot study recorded and sampled the decomposition process every two hours until the process was complete. The piglet cadavers from the summer and fall sampling periods were sampled three times a day to optimize the intervals. Decomposing tissue samples from the piglet cadavers were also collected to provide a correlation between the odors emitted from the maggots and the pig cadavers themselves. The findings include an assortment of chemical compounds emitted from each of the maggots collected exhibiting distinctive odor profiles as a determinant of the stage of decomposition.

#### Odor, Insect, Decomposition



### B32 Use of Blood Volatiles as Trace Evidence

*Lena M. Dubois, MSc\*, University of Liège, Liège 4000, BELGIUM; Katelynn A. Perrault, PhD, Chaminade University of Honolulu, Honolulu, HI 96816; Pierre-Hugues Stefanuto, PhD, University of Liège, Liège 4000, BELGIUM; Jean-François M. Focant, PhD, University of Liège, Liège 4000, BELGIUM*

**Learning Overview:** After attending this presentation, attendees will understand how Volatile Organic Compounds (VOCs) released from human blood can be used to indicate the time since deposition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a scientific basis for the use of blood VOC profiling as a tool to determine time since deposition and establishing a sequence of events.

The detection of blood at a crime scene can provide critical information about the nature of events that occurred, the order of events, and possibly the identity of the individuals involved. Therefore, the detection of blood has always been an important aspect of forensic investigations. Establishing an accurate VOC profile of blood can assist with developing and improving existing forensic methods to locate blood. This can be relevant at a crime scene or may also apply to the search for living and deceased individuals using scent detection canines or portable detection devices. In recent years, the possibility to train scent detection canines on blood has become more popular by forensic agencies. In order to provide more information about such applications, thermal desorption comprehensive two-dimensional gas chromatography (TD-GC×GC) coupled to time-of-flight mass spectrometry (TOFMS) with flow modulation and variable-energy electron ionization (EI) has been proposed as a novel analytical tool that can increase the ability to separate and identify blood VOCs to better understand which target molecules are involved in blood aging. Classical and soft EI (e.g. 70 eV and 14 eV) were applied to each sample in subsequent injections. This allowed effective mass spectral library-searching using classical EI spectra, while providing complementary soft EI spectra, with enhanced molecular ion and reduced fragmentation for confirmation of compound identity. In addition, the use of flow modulation in comparison to thermal modulation permitted the detection of compounds with low molecular weights, allowing the detection of compounds that may have been previously undetected in blood VOC research. The implementation of an internal standard mix allowed semi-quantitation of a subset of the detected compounds.

Statistical analysis such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) demonstrated that differences in blood VOC profiles due to sample aging surpassed inter-individual variations. The results further confirmed that distinctive patterns existed between fresh and aged blood, but also demonstrated that TD-GC×GC-TOFMS was able to highlight subtle differences in VOC profiles within the first week of aging. Improved understanding of the evolution of VOC profiles will help to improve the training of scent detection canines. In addition, the proposed method can be further developed to provide an analytical tool to indicate time since deposition. Such evidence can be crucial and can assist during crime investigation.

This research is significant because the study contributed to the exact determination of the blood VOC profile during degradation which can be used to improve scent detection canines training. Furthermore, it was demonstrated that monitoring blood VOC profiles can indicate status of degradation, related to the time since deposition, which can serve as trace evidence providing crucial investigative information during crime investigation.

#### **Blood Degradation, Volatile Organic Compounds (VOCs), Trace Evidence**



### B33 The Use of Inductively Coupled Plasma-Mass Spectrometry (ICP/MS) in the Identification of the Elemental Composition of Kratom and the Determination of Geographical Origin

Cody L. Braley\*, Parkville, MD 21234; Ellen Hondrogiannis, PhD, Towson University - Department of Chemistry, Towson, MD 21252

**Learning Overview:** After attending this presentation, attendees will understand the principles of Inductively Coupled Plasma-Mass Spectrometry (ICP/MS) and how the elemental composition of kratom can be used in conjunction with Discriminant Function Analysis (DFA) to classify kratom samples by geographical origin.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new method to differentiate kratom based on elemental composition with the purpose of increasing the discrimination between visually similar kratom samples and helping with geographical origin attribution.

Kratom (*Mitragyna speciosa*) is a tropical evergreen tree native to Southeast Asia whose leaves are commonly consumed for their analgesic, opiate-like, and stimulant properties. The most common alkaloid that produces these effects is mitragynine, although there are over 40 alkaloids produced by the plant. Kratom is indigenous to Thailand, Indonesia, Malaysia, and Vietnam. When Kratom is consumed in high doses, a depressant effect due to mitragynine is observed and has been found in a number of fatalities in combination with other CNS depressant drugs.

In recent years, kratom's use as a recreational drug has increased worldwide and numerous case studies have documented the various effects on users. Kratom is currently a controlled substance in 16 countries including Malaysia, Myanmar, and Thailand. The United States Drug Enforcement Administration (DEA) currently includes kratom on the list of Drugs and Chemicals of Concern and is considering labeling kratom as a Schedule I substance. The legality of kratom in the United States currently varies by state. In February 2018, the Food and Drug Administration (FDA) stated that there is no evidence that kratom is safe or effective for treating any condition.

Elemental composition determination is beneficial as it can be utilized for geographical origin attribution. The basis of geographical origin determination is that kratom grown in different regions will have variations in trace elements due to soil nutrients and composition, water content, and fertilizer use. This allows for the determination of the geographical origin of unknown kratom samples.

In this study, 33 kratom samples were purchased from 4 reputable internet vendors with a varying number of countries of origin. The countries of origin include Malaysia, Thailand, Borneo, Bali and Vietnam. The samples were digested and then analyzed using ICP-MS to determine the concentration of 21 different elements. Discriminant Function Analysis (DFA) was used to analyze the resulting elemental composition for each kratom sample and identify elements that optimized the multivariate differentiation of each sample group.

#### Kratom, ICP/MS, Attribution



### **B34 A Casework Review for Determining Time Since Intercourse in Boston, Massachusetts**

*Cassandra A. Swart, BS\**, Boston University School of Medicine, Boston, MA 02118; *Caitlin E. Rogers, MS*, Colorado Bureau of Investigation, Pueblo West, CO 81007; *William Cavedon, MS*, Boston University School of Medicine, Boston, MA 02118; *Amy N. Brodeur, MFS*, Boston University School of Medicine, Boston, MA 02118; *Kathryne Hall, MS*, Boston Police Crime Laboratory, Boston, MA 02120

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**Learning Overview:** After attending this presentation, attendees will be able to comprehend and utilize a reliable framework for estimating time since intercourse in living victims.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the estimation of time since intercourse.

Time since intercourse (TSI) is the approximate time elapsed between an alleged sexual assault and the collection of a Sexual Assault Evidence Collection Kit (SAECK) from a victim. The estimation of TSI, or Post Coital Interval (PCI), can be crucial information for particular cases in which the time between offense and the collection of a SAECK is in question. Often, forensic scientists must evaluate the significance of biological evidence in SAECKs, but the variability in current literature complicates interpreting these results. Developing a method to estimate TSI based on a more extensive review of forensic casework would provide investigators with a fundamental tool for estimating a general timeline in which the offense occurred. This information may play an important role in supporting or refuting a narrative or weighing the significance of the evidence at hand.

This study aims to develop a reliable framework for estimating TSI in living victims based on casework received by Boston Police Department Crime Laboratory, Boston, MA. Additionally, this study seeks to determine if any statistical significance exists between the victim's reported post-coital activities and the collection of evidence, including the presence of intact sperm cells.

The need to expand research on estimating TSI for sexual assault victims using actual forensic casework is crucial to provide a more reliable method for TSI estimation, compared to previous studies, which have generally been based on fertility studies. Between the years of 2009 and 2017, over 1,800 reported SAECKs were submitted to the Boston Police Department for evidence processing. Approximately 500 of these kits tested positive for the identification of sperm cells during original kit processing. More than 250 cases met the qualifications for this study, including a living victim, smear slides prepared by a medical professional, and the identification of sperm cells. To estimate TSI, the smear slides from these cases were microscopically examined for the presence of intact sperm cells with the aid of Kernechtrot Picroindigocarmine (KPIC) stain.

Preliminary results indicate that the observation of intact sperm on vaginal smear slides rarely surpasses a TSI of 25 hours, with an average of 12 hours. Furthermore, preliminary results indicate that the observation of intact sperm on anal and oral smear slides rarely surpasses a TSI of 15 hours, with an average of 6 hours, and 3.75 hours, with an average of 3.75 hours, respectively. This study provides reliable evidence based on actual casework samples for more accurately estimating the time since intercourse in living victims of sexual assault crimes.

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#### **Time Since Intercourse (TSI), Sexual Assault Collection Kit, Post-Coital Interval (PCI)**

### **B35 A Strategic Design to Arrest a Serial Rapist and Killer: The Role of a DNA Profiling Database and Population Census Data**

Mohammad A. Tahir, PhD, Punjab Forensic Science Agency, Lahore, Punjab, PAKISTAN; Syed W. Hamdani\*, Punjab Forensic Science Agency, Lahore, Punjab 53700, PAKISTAN; Mudassar Naseer, MPhil, Punjab Forensic Science Agency, Lahore 53700, PAKISTAN; Nasir Siddique, MPhil, LLB, Punjab Forensic Science Agency, Lahore, Punjab 53700, PAKISTAN

**Learning Overview:** After attending this presentation, attendees will be able to understand the strategic design used by the investigation agency to arrest a serial rapist and killer reported in the Kasur district in the Punjab province of Pakistan. The case study will be helpful to understand the process where an unsolved serial rape and murder case was solved and the culprit was brought to justice using modern forensic tools. Moreover, this case study will help to understand the raping trends with minors in the area.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by providing the results of the case studies which were concluded based on population census data of the Kasur district of Pakistan. Attendees will also be informed about the admissibility of DNA profiling and digital evidence in the court of law in Pakistan, the increasing confidence in forensic tools after this case, and the increasing trend of utilizing forensic expertise rather than conventional investigation methods (e.g., eyewitnesses, etc.).

Since 2013, nearly 10,620 rape cases of minor girls were reported in Pakistan against which only 112 convictions were obtained in courts of law. Such low conviction rate is due to poor investigative strategy and use of old conventional investigative tools. In recent studies it has been observed that 9 girls and 3 boys out of every 100 persons are victims of child abuse.<sup>1</sup> In this case study first rape and murder was reported in April 2015, after which a series of cases were reported. The last case was reported on January 9, 2018. In a span of 3 years, a total of 8 cases of rape came into notice in which minor girls (average age 9 years) were abducted from outside their houses. Out of 8 victims, 5 were murdered after rape and their dead bodies were found mostly in houses under construction, vacant plots, or parks in the vicinity of abduction. All of these incidents took place in a radius of 4 kilometers with 2-3 months' time intervals.

Analysis of case samples in DNA and serology section of Punjab Forensic Science Agency (PFSA) revealed the single male profile from all the victims. It was a matter of grave concern, and in first time in the history of Pakistan a strategy was devised to reach this serial killer. Police forces, intelligence agencies, PFSA and regional police heads were called together to plan the strategy. Population census data was released for the first time to the civil administration with the permission of the prime minister of Pakistan to locate the suspect. The targeted average age group was selected as 20 to 35 years based on the physical appearance of the suspect from CCTV footages received from the locality of incident. The visual images were enhanced by the audio-visual department of PFSA. Buccal swabs of 1,174 individuals were collected by door-to-door searches and verified from census data. DNA extraction was performed employing organic phase separation. DNA from the buccal swab standards of all the suspects was characterized through the Polymerase Chain Reaction (PCR) at the genetic loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and Amelogenin using AMPFISTR® Identifiler® Plus amplification kit. The amplified products were run on the ABI 3500 Genetic Analyzer and the results obtained were analyzed using GeneMapper® ID-X Software Version 1.2.

The DNA profile of suspect (sample No. 814) matched with the DNA profile of the perpetrator that was already maintained in the PFSA DNA database. The DNA profile of suspect No. 814 was previously obtained from the sperm fractions of vaginal/anal swabs and semen stains found on the clothing of the 8 minor girls. Based on DNA analysis, 90 suspects apprehended by the police and 1,173 individuals whose samples were collected during the mass screening conducted by PFSA, were eliminated as being possible source of foreign male DNA obtained from all the eight minor victims.

#### **Reference(s):**

1. Barth, J., Bermetz, L., Heim, E., Trelle, S., Tonia, T. (2013). The current prevalence of child sexual abuse worldwide: a systematic review and meta-analysis. *International Journal of Public Health*, 58: 469-483.

#### **DNA, Serial Killer, Census Data**



### **B36 The Recovery of Foreign DNA Introduced Through Kissing**

*Joyce P. Williams, DNP\*, Randallstown, MD 21133; David A. Williams, DDS\*, Randallstown, MD 21133*

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**Learning Overview:** The goals of this presentation are to: (1) describe the prevalence of oral sexual assault and understand the time frame DNA lasts in the mouth; (2) explain the differences in collection locations in the mouth; and (3) recommend improvements in oral evidence collection protocols.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the competence level of persons recovering foreign DNA in and around the oral cavity following sexual violence encounters, thus enhancing the overall outcomes for victims.

Prosecution of cases with analyzed forensic evidence (including DNA) are known to increase conviction rates, but unless there is a scientific basis for the evidence to be admitted in Court under the *Daubert* Rule, evidence that links a perpetrator to a victim may be excluded.

Victims subjected to oral rape may have foreign DNA deposited in the mouth. However, there is no scientifically based protocol for the effective collection of a suspect's DNA from the oral cavity whether by a forensic practitioner, other medical provider, or law enforcement. Different jurisdictions vary with respect to their data collection including device used, area of collection, and laboratory procedures. Collection devices and laboratory procedures have been researched, but the areas of the mouth where the collection takes place have not been studied.

A review of the literature to examine protocols for collection including the National Protocol for Sexual Assault Medical Forensic Examinations only provided recommendations for collection.

Scientific protocols for the collection of DNA on the skin and in the vagina have provided rigor regarding collection techniques using the double swab and Y-STR respectively.

There is a need for empirically-based evidence to determine sensitive and probable areas to swab within the oral cavity and the number of swabs to be collected.

The evolution of DNA analysis is now more sensitive and specific to identify perpetrators.

Gaps exist in the collection of evidence from the oral cavity. This study will describe the research involved to distinguish areas of higher concentration in and around the oral cavity where male DNA can be collected. The rationale for the study is to isolate areas where higher concentrations of male DNA reside following intimate contact and to improve the efficiency of protocols for the collection process. Saliva is recognized as an important source of DNA.

The results may improve educational training for all professionals who provide expert care to victims of violence (forensic nurses, emergency providers, and law enforcement). Lab analysts could receive evidence that is labeled from precise areas in and around the mouth to examine and identify foreign DNA. Additionally, this study may improve the national standard for the collection of foreign DNA in and around the oral cavity in sexual assault cases.

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**DNA, Oral Assault, Offender**



### B37 Rapid DNA Turnaround for the Analysis of All Victim Sexual Assault Evidence When a Kit Is Collected

*Diana K. Faugno, MSN\*, Palm Desert, CA 92211; Patricia M. Speck, DNSc, Hoover, AL 35226*

**Learning Overview:** After attending this presentation, attendee's will be able to: (1) define Rapid DNA Service (RADS) and its scope; (2) be able to discuss program costs and budget; and (3) describe the implementation process for the forensic nurse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with the knowledge to be able to compare this program's success in handling the backlog of sexual assault kit analysis to their own programs. The victims also benefit by knowing their kit is being reviewed in a timely manner.

In May 2011, the California Department of Justice, Bureau of Forensic Services, Jan Bashinski DNA Laboratory launched a pilot project in four counties—Marin, Solano, Napa, Sonoma—to test a portion of all victim sexual assault kits within 30 days. This project, known as the Rapid DNA Service (RADS), was found to be effective and sustainable. RADS is effective because a rapid analysis provides the best circumstances for rapidly solving a case, it prevents future crimes by rapidly removing predators from the community, and counties participating in this program no longer accrue future backlogs. RADS is sustainable because 95% of the time, the turnaround time goal has been successful. With the success of the pilot project, combined with the implementation of the Sexual Assault Victim's DNA Bill of Rights (AB 1517) in 2016, the California Department of Justice has elected to roll out the RADS program to the 46 counties serviced by the Bureau of Forensic Services. As of March 2017, RADS has expanded to five of the state's DNA laboratories and 28 counties. Over 2,000 cases have been processed resulting in over 700 cases with searchable profiles, of which over 300 have hit to a known offender or arrestee within the CODIS (Combined DNA Index System) database. Thirty-five percent of RADS cases result in a searchable CODIS profile, and 44% of the time, those profiles hit to a known individual. The RADS work flow requires additional steps for the Sexual Assault Forensic Nurse (SAFN) to perform during the sexual assault medical forensic examination. Implementation of this process involves initial training for the SAFN and continued communication. A monthly report is sent to the program coordinator with the case numbers listed as well as if there was a hit on any of the swabs FedEx to the crime lab. A forensic nurse from Riverside County, CA, will discuss cases with searchable CODIS profiles developed from RADS samples as well as several barriers for the nursing staff when executing this program.

Further Information, please visit the following websites:

[www.fbi.gov/about-us/lab/biometric-analysis/codis/rapid-dna-analysis](http://www.fbi.gov/about-us/lab/biometric-analysis/codis/rapid-dna-analysis)

[www.fbi.gov/about-us/lab/biometric-analysis/codis/codis-and-ndis-fact-sheet](http://www.fbi.gov/about-us/lab/biometric-analysis/codis/codis-and-ndis-fact-sheet)

[www.fbi.gov/about-us/cjis/fingerprints\\_biometrics/ngiswgdam.org/](http://www.fbi.gov/about-us/cjis/fingerprints_biometrics/ngiswgdam.org/)

[www.washingtonpost.com/wp-dyn/content/article/2010/03/06/AR2010030602500.html](http://www.washingtonpost.com/wp-dyn/content/article/2010/03/06/AR2010030602500.html)

**Sexual Assault Evidence Collection, DNA, Forensic Nurses**



### B38 Efficient Sampling of Skeletonized Human Crania for DNA Testing

Suni M. Edson, MS\*, Armed Forces DNA Identification Laboratory, Dover Air Force Base, DE 19902

**Learning Overview:** After attending this presentation, attendees will understand how to apply an effective cranial sampling protocol in their practices. The results of this study are applicable to any laboratory performing human identification on skeletonized remains.

**Impact on the Forensic Science Community:** This presentation will impact the forensics community by providing information on how best to sample skeletonized human crania for DNA, allowing for an increased degree of success for Human Identification (HID).

DNA testing of skeletonized human remains continues to be a challenging task in the field of human identification. Efficient selection of elements from the remains tends to provide the best foundation for success, regardless of the extraction technique used or the DNA platform being tested. In mass fatality events involving highly commingled remains, the most numerous element establishes the minimum number of individuals present. In practice, DNA profiles from cranial and post-cranial remains are desired to provide a re-association, as proper re-articulation can be difficult.

This presentation will examine the success rates of each platform tested as compared to each cranial element and provide guidance to the laboratory practitioner on some of the best practices for the sampling of cranial remains for DNA testing. In this study, 2,177 cranial elements were examined during regular casework done at the Armed Forces Medical Examiner-Armed Forces DNA Identification Laboratory (AFMES-AFDIL) from 1990-2018. These samples were provided as part of DNA testing done in partnership with the Defense POW/MIA Accounting Agency (DPAA). Elements processed have postmortem intervals ranging from approximately 45-78 years. Teeth were not included in the survey and will be addressed in a separate study.

The purpose of this project was to develop a recommendation that would be wide-ranging and applicable to remains found in numerous burial conditions, as well as to laboratories that might use different processing techniques. Among the remains tested were those recovered from disinterments, *in situ* locations, and curated remains. Testing involved three different extraction protocols: organic; complete demineralization plus organic purification (Demin1); and complete demineralization plus inorganic purification (Demin2). In addition, multiple types of DNA profiles were generated: mitochondrial DNA (mtDNA) via Sanger sequencing and Next Generation Sequencing protocols (NGS); Y-STR profiles from an enhanced AmpFISTR® Yfiler™ protocol; and multiple STR kits: AmpFISTR® MiniFiler™, AmpFISTR® Identifiler™, and PowerPlex® Fusion.

The occipital is the most commonly sampled element of the cranium, with 687 tests being performed. MtDNA Sanger sequencing of occipital samples extracted with complete demineralization and organic purification have a 91% success rate. While this seems to be a high rate of success, nearly all cranial samples extracted with the same method have a 91-92% success rate, except for cranial fragments of unspecified origin, which have a 66% success rate. If the extraction method is not considered, occipital and temporal fragments are equally successful for mtDNA Sanger sequencing at 85%.

The cranial element that consistently provides results across any platform tested is the temporal. An initial survey of cranial samples tested at AFMES-AFDIL found similar results.<sup>1</sup> However, only mtDNA Sanger sequencing and two types of extractions were evaluated.

Attendees should be able to take this information back to their laboratories and apply an effective cranial sampling protocol to their practices. The results of this study are applicable to any laboratory performing human identification on skeletonized remains.

#### Reference(s):

- <sup>1</sup>. Suni M. Edson, Alexander F. Christensen, Suzanne M. Barritt, Audrey Meehan, Mark D. Leney, and Louis N. Finelli, "Sampling of the cranium for mitochondrial DNA analysis of human skeletal remains," *Forensic Science International: Genetics Supplement Series 2*, vol. 1 (2009): 269-270, <https://doi.org/10.1016/j.fsigss.2009.09.029>.

#### Human Identification (HID), Skeletonized Remains, DNA





### B39 Rapid DNA Analysis for Disaster Victim Identification in New York City

*Andrew J. Schweighardt, PhD\**, Northport, NY 11768; *Veronica N. Cano, BS*, Office of the Chief Medical Examiner, New York, NY 10016; *Yasser D. Hernandez, BA*, Office of the Chief Medical Examiner, New York, NY 10016; *Arianna Lionetti*, Westwood, NJ 07675; *Brad Greenstein, MS*, Office of the Chief Medical Examiner, New York, NY 10016; *April V Kwong, MSc*, Office of the Chief Medical Examiner, New York, NY 10016; *Kaleel Wainwright, MPH*, Office of the Chief Medical Examiner, New York, NY 10016; *Carl Gajewski, MS*, Office of the Chief Medical Examiner, New York, NY 10016; *Mark A. Desire, MS, JD*, Office of the Chief Medical Examiner, New York, NY 10016

**Learning Overview:** Mass disasters that result in human casualties require the need for swift and accurate victim identification. Visual identification, fingerprinting, and dental comparisons are often precluded by the poor condition of human remains recovered at a disaster site. DNA testing, although historically expensive and time-consuming, may sometimes be the only pathway to identification. For example, DNA testing played a role in almost 90% of identifications made from the World Trade Center attack in 2001. After attending this presentation, attendees will gain insight into the ability of Rapid DNA to enhance identification capabilities in the event of a mass fatality.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the way victim identification is handled by the forensic science community.

Rapid DNA systems encompass the stages of DNA testing such as extraction, amplification, electrophoresis, and analysis. All phases of testing are carried out in the self-contained unit. These systems are designed to be field deployable by military and law enforcement personnel and are therefore often rugged and easily transported. Instrument operation is fully automated and requires no user intervention after sample input. Typical processing time is less than two hours from the time the sample is loaded. Legislation enacted within the past two years has enabled NDIS-accredited laboratories to process DNA samples and search the resulting profiles in a database. A rapid DNA system would be ideally suited for a mass disaster because of its ability to function outside the typical laboratory setting and because of the speed with which results are obtained.

Postmortem bone samples from non-casework autopsies were tested using the ANDE™ Rapid DNA Analysis System. These bones had been previously extracted using the bone protocol routinely performed by the New York City Office of the Chief Medical Examiner (OCME) and amplified with the Identifiler™ kit. Bones were divided into groups and prepared using either the manufacturer's instructions or the OCME's bone protocol. Samples prepared and extracted using both methods were tested by Rapid DNA analysis. The results obtained using the conventional methods were compared to those obtained by Rapid DNA analysis.

Severely damaged bones collected from the site of the Twin Towers collapse that have yet to be identified still exist. Successful DNA typing of these samples has been prevented by the damage caused by exposure of the bones to fire, heat, and jet fuel at the site of the disaster. A select group of bones collected from the site of the collapse which have previously yielded no DNA profiles were tested using Rapid DNA analysis to determine if the results displayed any improvement over conventional protocols.

The success of any DNA identification effort relies upon the submission of reference samples. Many of the reference samples submitted for the World Trade Center disaster were tested with an amplification kit that is no longer used by the OCME because it does not contain enough loci to generate CODIS-eligible profiles. The OCME has recently converted to a newer kit with more loci to comply with FBI requirements for CODIS participation. Many of the reference samples collected have yet to be re-tested with the newer kit to facilitate better comparisons. A group of reference samples submitted for the World Trade Center identification effort was selected for Rapid DNA analysis to demonstrate that this technology can provide DNA profiles that more closely align with current amplification kits. Rapid DNA analysis may eventually be used to re-test all 9/11 reference samples to enable better comparisons.

Rapid DNA can significantly reduce the time commitment needed for processing postmortem samples and analyzing the resulting DNA profiles. Simultaneous typing of reference samples enables fast comparisons to occur so that results can be reported with utmost speed and without compromising reliability. Rapid DNA analysis has the capability to drastically improve the overall success of disaster victim identification.

#### Rapid DNA, Disaster, Bone



### **B40 Coupling DNA and Gas Chromatography/Mass Spectrometry (GC/MS) Analysis of Skeletal Remains: A Case Study of the USS *Oklahoma***

*Suni M. Edson, MS\*, Armed Forces DNA ID Laboratory, Dover Air Force Base, DE 19902*

**Learning Overview:** The purpose of this presentation is to provide information on a novel application of Gas Chromatography/Mass Spectrometry (GC/MS) analysis that could improve the speed at which persons are identified in mass fatality incidents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing a novel application of GC/MS and how this data may be coupled with DNA testing results from skeletonized human remains.

The Armed Forces Medical Examiner System-Armed Forces DNA Identification Laboratory (AFMES-AFDIL) partners with the Defense POW/MIA Accounting Agency (DPAA) to provide DNA testing of skeletonized human remains of missing service members from past military conflicts. Most commonly, the remains found are not those of a single individual, but comprise commingled individuals from aircraft crashes, ground losses, ships, or other incidents. One of the largest single assemblages of remains ever analyzed is that of the remains of the individuals killed-in-action on the USS *Oklahoma*.

On 7 December 1941, torpedoes sank the USS *Oklahoma* during an attack on Pearl Harbor, HI. In the process of sinking, the ship rolled over and then remained inverted in the harbor until 1943. The ship was righted at that time and the remains of approximately 429 individuals were recovered and placed in group burials. Efforts to identify the remains made in the late 1940s resulted in 35 persons being buried as individuals. The remainder were buried in 46 plots at the National Memorial Cemetery of the Pacific (NMCP) in Hawaii.

Beginning in 2003, the DPAA initiated an effort to identify the 394 individuals from the graves at the NMCP. The testing of the first casket to be disinterred generated over 100 different mitochondrial DNA (mtDNA) profiles. The remaining 45 caskets were disinterred in 2015, and to date nearly 5,000 osseous fragments have been submitted to AFMES-AFDIL for mtDNA testing, and approximately 3,000 have been completed.

As part of the DNA extraction protocol at AFMES-AFDIL, the exterior of the bone is removed using a sanding tool. For the USS *Oklahoma* samples, the detritus was often sticky with the residue of fuel oil and adipocere. Rather than being discarded, this material was collected from a selection of samples by the analysts and retained as part of a study on the carry-over of inhibitory materials from skeletal materials to the associated extracted DNA.

The skeletal detritus from 208 individual elements was treated sequentially with acetonitrile and dichloromethane. The eluate was removed and allowed to evaporate in an effort to concentrate materials found in the osseous elements. The concentrated materials were then re-suspended in methanol for injection on an Agilent 7890A-5975C GC/MS. Contained within the skeletal materials were products of decomposition, such as fats and esters, fat-soluble medications, and chemicals inherent to the ship itself, such as anthracene and other fuel oil components.

It was expected that the remains would show largely the same chemical profile, especially with regards to the fuel oil present. However, this was not found to be so. The GC/MS traces showed fuel profiles consistent with what should have been on the ship, the USS *Oklahoma* being one of the first ships to be powered by fuel oil. However, they were not all the same. In between the peaks of fuel and decomposition by-products were traces of medications and other chemicals. When the GC/MS data from the different solvents were compiled and paired with the DNA extracted from the remains, it was found that the generated chemical profiles were unique to the individual.

While GC/MS analysis of skeletonized human remains still needs to be perfected, it has far-reaching implications for human identification. Coupling DNA testing with GC/MS profiling could greatly improve the speed with which large assemblages of commingled remains can be sorted. Once a GC/MS profile is paired with the DNA profile, one only needs to test skeletal elements in GC/MS, which is a markedly faster and cheaper method than DNA testing and requires very little training.

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#### **Skeletonized Remains, DNA, GC/MS**

### B41 The Development of an Innovative Massively Parallel Sequencing (MPS) Panel of Microhaplotypes for Improved Biogeographic Ancestry Inference

Sathya Prakash Harihar\*, The George Washington University, Department of Forensic Science, Washington, DC 20007; Fabio Oldoni, PhD, The George Washington University, Washington, DC 20007; Aishwaryaa Subramanian, The George Washington University, Washington, DC 20052; Leena Yoon, Tysons, VA 22102; Sharon C. Wootton, PhD, South San Francisco, CA 94080; Robert Lagacé, BS, Thermo Fisher Scientific, South San Francisco, CA 94080; Ryo Hasegawa, BS, Foster City, CA 94404; Joseph P. Chang, BS, Thermo Fisher Scientific, South San Francisco, CA 94080; Moses S. Schanfield, PhD, The George Washington University, Department of Forensic Sciences, Washington, DC 20007; Kenneth Kidd, PhD, Yale University School of Medicine, New Haven, CT 06520; Daniele S. Podini, PhD, Washing State University, Department of Forensic Science, Washington, DC 20007

**Learning Overview:** After attending this presentation, attendees will be able to understand the potential of using microhaplotype (MH) markers for ancestry inference.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the usefulness of an innovative massively parallel sequencing (MPS) assay of 74 MH loci for enhancing biogeographic ancestry prediction capabilities.

With the advent of high-throughput sequencing technology new genetic markers called microhaplotypes (MHs) have become available to the forensic community. MHs are loci characterized by the presence of two or more single nucleotide polymorphisms (SNPs) within a short distance from each other (< 300 nucleotides) and associated in three or more allelic combinations.<sup>1</sup> Standard Sanger sequencing method is unable to determine the *cis/trans* relationship among SNP alleles within the same expanse of DNA. MPS instead allows distinguishing the parental haplotypes at any given locus within the same amplicon by specific clonal sequencing of each individual DNA strand. This process allows determining the haplotype phase of the targeted SNP alleles within each MH locus. Key characteristics of MHs include multi-allelic nature, absence of stutter peaks, small amplicon size, and lower mutation rates than conventional short tandem repeat polymorphisms (STRPs).<sup>2</sup> All of these features contribute to making MHs promising candidates for different forensic applications including ancestry inference, mixture deconvolution, and human identification. The aim of this study was to evaluate the performance of a recently developed MPS-based MH assay for the prediction of biogeographic ancestry of individuals using specifically phased-inferred allele frequencies reported on the Allele Frequency Database (ALFRED) database.<sup>3,4</sup>

A novel panel targeting 74 MH loci and totaling 230 SNPs was developed on the Ion Chef™ and Ion S5™ MPS (Thermo Fisher Scientific) platform.<sup>5</sup> A set of 20 unknown African Americans (AAs), European-Americans (EAs), East Asian Americans (EAAs), South West Hispanics (SWHs) and one Mexican Pima (MP) test-samples were selected and tested for evaluating biogeographic origin prediction. Statistically phased-inferred allele frequencies of the selected 74 multi-SNP loci were extracted from ALFRED and further utilized to calculate the random match probability (RMP) of the unknown test-samples in each relevant population. The Log<sub>10</sub> of the RMP was further calculated for all test-samples by utilizing ALFRED allele frequencies from the 74 MH loci across a worldwide set of 26 different populations representative of African (7), European (9), Asian (6) and Native American (4) population clusters. Overall, the biogeographic ancestry of the 80-unknown test-population samples was correctly predicted using the ALFRED MH allele frequencies. Among the populations studied, the Log<sub>10</sub> RMP calculated for each test-sample was found to average significantly higher in the corresponding population of origin while SWHs averaged equally high in European and Native American populations, as expected. Average values of Log<sub>10</sub> RMP for unknown AAs, EAs, EAAs, SWHs test-samples ranged between -69 and -78, -63 and -70, -62 and -65, -76 and -83 across African, European, East Asian and European/Native American populations, respectively while Log<sub>10</sub> RMP of MP test-sample ranged between -56 and -60 in NA populations.

These findings indicate that the MPS panel of 74 MH loci is an effective forensic DNA tool, which provides valuable information on the biogeographic origin of individuals complementing the accuracy of current available forensic DNA-based ancestry prediction assays.

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1. Pakstis AJ, Fang R, Furtado MR, Kidd JR, Kidd KK. Mini-haplotypes as lineage informative SNPs and ancestry inference SNPs. *European Journal of Human Genetics* (2012) 20(11): 1148-1154.
2. Kidd KK, Speed WC, Pakstis AJ, Podini DS, Lagacé R, Chang J, Wootton S, Haigh E, Soundararajan U. Evaluating 130 microhaplotypes across a global set of 83 populations. *Forensic Science International: Genetics* (2017) 29:29-37.
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#### Microhaplotype, MPS, Ancestry Prediction



### B42 The Persistence of Salivary $\alpha$ -Amylase on Decomposing Skin

Jennifer P. Kidd\*, Arizona State University, Phoenix, AZ 85306; Martin Jimenez-Navarro, Arizona State University, Phoenix, AZ 85306; Courtney Manas, Arizona State University, Phoenix, AZ 85306

**Learning Overview:** After attending this presentation, attendees will better understand the factors that may affect the persistence of saliva evidence over time and will have a more comprehensive timeline of how long saliva is detectable on a decomposing body.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing a timeline during which saliva will be detectable on a body and detailing how the conditions of the body may impact the ability to detect saliva. The timeline may empower investigators to determine if saliva is likely to be found on the body or not, based on the estimated time of death and state of the body. If the detection of saliva is not possible, investigators may pursue other evidence items for testing and potential recovery of DNA.

This study explores the hypothesis that environmental factors impact the ability to detect saliva and suggests that it possible to establish a timeline in which investigators can expect to be able to detect saliva.

Crime scene investigators have many types of evidence at their disposal, and biological fluids are among the most useful of these. Some forms of biological evidence, like blood and semen, have been thoroughly researched while other fluids, such as saliva, are much less common in research.<sup>1</sup> Saliva evidence may be found at a variety of crime scenes including homicides and sexual assaults, which makes the understanding of this evidence essential for investigators and forensic scientists.

The research performed aims to address the lapse in research regarding saliva evidence in general with a focus on its persistence over time. The experiment observed the ability to detect salivary  $\alpha$ -amylase over time on decomposing skin. The study used a total of 33 subjects which were divided into three experimental groups. Each group was designed to reflect standard conditions that a deceased person may be found in, with each environment varying in temperature and exposure to environmental factors. Saliva samples were collected from the bodies and tested for the presence of salivary amylase using the SALiGAE<sup>®</sup> indicator test.

The results suggest that environment has a significant impact on the ability to detect saliva over time. The data also demonstrates that the detection of saliva may be possible for a longer period of time than previously suggested in other research.<sup>2</sup>

This presentation will detail an experiment in which saliva was deposited onto decomposing bodies to determine if saliva is detectable over time and evaluates the impact of environment on the persistence of saliva.

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1. Baxter, & Rees. (1975). The identification of saliva in stains in forensic casework. *Medicine, Science and the Law*, vol. 15, no. 1, 1975, pp. 37–41., Retrieved from doi:10.1177/002580247501500108.
2. Dockery, Kenna, McDermott, McKenna, & Smyth. (2010). The recovery and persistence of salivary DNA on human skin. *Journal of Forensic Sciences*, vol. 56, no. 1, Dec. 2010, pp. 170–175., doi:10.1111/j.1556-4029.2010.01520.x.

**Saliva, Persistence, SALigAE**



**B43 WITHDRAWN**



### **B44 The Development of a Single-Tube Assay for the Simultaneous Detection of Blood, Semen, and Saliva Utilizing DNA Methylation and ScreenClust® High Resolution Melt Software**

Ellyn A. Zeidman, BS\*, Towson, MD 21252; Cynthia B. Zeller, PhD, Towson University, Towson, MD 21252

**Learning Overview:** After attending this presentation, attendees will be familiar with methylation-specific Polymerase Chain Reaction (PCR) and High-Resolution Melt analysis (MSP-HRM) and the utility of this method for detection of blood, semen, and saliva, individually and in combination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing forensic scientists to use a single DNA extract to obtain serological test results as well as a genotype, reducing the overall amount of sample and analyst time required. This multiplex assay will allow quick, straightforward, and simultaneous serological determination of bodily fluids.

Using MSP-HRM analysis, blood, semen, and saliva were differentiated. To identify bodily fluids in this manner, tissue-specific methylation differences are targeted.<sup>1</sup> To do this, extracted DNA samples were bisulfite-treated, causing the unmethylated cytosines in DNA to be converted to uracils. These uracils will then be converted into thymines during PCR, lowering the melting temperature of the resulting amplicon due to the decreased guanine and cytosine content in the DNA. During the bisulfite treatment, methylated cytosines in the sequence will remain the same, thus exhibiting a higher melting temperature.

Previously published studies have found specific regions of DNA that are differentially methylated in forensically relevant bodily fluids.<sup>2-6</sup> Using these previously described primers such as BCAS4, DACT1, DDX4, DPPA5, and ZC3H bodily fluids were able to be distinguished from one another in a multiplex reaction due to the differences in melt temperatures. These melt profiles were then statistically separated using the ScreenClust software, which performs principle component analysis. This software examines the differences in the melt profiles to distinguish the components of a sample.

This research has been able to discriminate between the bodily fluids individually as well as in various combinations. Additionally, this protocol has provided the ability to identify bodily fluids with as little as 2.0 ng of DNA. The study has been shown to work on samples of blood, semen, and saliva from different individuals.

Currently the assay is being performed using mixtures of bodily fluids with differing ratios of DNA extract to help establish a limit of detection. Casework-like samples of mixed bodily fluids are prepared and analyzed using this method. This involves mixtures of bodily fluids themselves as opposed to mixtures of the extracts to determine if the assay remains consistent. This will be conducted as a single-blind study to remove bias during the analytical process. It is anticipated that the results of this analysis will conclude in an assay that is cost-effective, efficient, and reliable, which can be incorporated into the current forensic DNA workflow using instrumentation commonly found in forensic laboratories.

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2. Fachel C., Quarino L., and Karnas K.J. High resolution melt curve analysis based on methylation status for human semen identification. *Forensic Sci Med Pathol*. 2017; 13: 86-91.
3. Madi T., Balamurugan K., Bombardi R., Duncan G., and McCord B. The determination of tissue-specific DNA methylation patterns in forensic biofluids using bisulfite modification and pyrosequencing. *Electrophoresis*. 2012; 33: 1736-1745.
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#### **Body Fluid Identification, High Resolution Melt Analysis, ScreenClust**

### B45 The Evaluation of a Novel Massively Parallel Sequencing (MPS) Panel of 74 Microhaplotypes for Ancestry Prediction of Four Major United States Population Groups

Aishwaryaa Subramanian\*, The George Washington University, Washington, DC 20052; Fabio Oldoni, PhD, The George Washington University, Washington, DC 20007; Sathya Prakash Harihar, The George Washington University, Washington, DC 20007; Leena Yoon, Tysons, VA 22102; Sharon C. Wootton, PhD, South San Francisco, CA 94080; Robert Lagacé, BS, Thermo Fisher Scientific, South San Francisco, CA 94080; Ryo Hasegawa, BS, Foster City, CA 94404; Joseph P. Chang, BS, Thermo Fisher Scientific, South San Francisco, CA 94080; Moses S. Schanfield, PhD, The George Washington University, Washington, DC 20007; Kenneth Kidd, PhD, Yale University School of Medicine, New Haven, CT 06520; Daniele S. Podini, PhD, The George Washington University, Washington, DC 20007

**Learning Overview:** After attending this presentation, attendees will better understand the advantages of using microhaplotype (MH) markers in addition to Single Nucleotide Polymorphism (SNP) and Insertion Deletion (InDel) panels for predicting the biogeographic ancestry of individuals.

**Impact on the Forensic Science Community:** This presentation will contribute to the forensic science community by providing an innovative MPS panel of MH loci, for improved ancestry prediction.

Recent advancements in high-throughput sequencing technologies have enabled exploring a new type of genetic marker: microhaplotypes (MHs).<sup>1</sup> These new forensic DNA markers are based on two or more SNPs within less than 300 bp from each other and can be genotyped using MPS platforms. The conventional Sanger sequencing method does not allow determining the *cis/trans* relationship of the SNP alleles (i.e., phase) within the same amplicon while MPS enables distinguishing the parental haplotypes by clonal sequencing of each individual strand of DNA, thus providing unambiguous SNP phase information at each locus. The small amplicon size, absence of stutter peaks along with lower mutation rates than conventional short tandem repeat (STR) loci are features that make MHs a promising marker for addressing relevant forensic challenges including ancestry prediction and mixture deconvolution.<sup>2</sup> In this study, the authors generated allele frequency databases for four American population groups and explored the potential for MPS-based MH analysis to provide biogeographic ancestry information.

A novel forensic panel of 74 MH loci was developed and implemented on the Ion Chef™ and Ion S5™ MPS (Thermo Fisher Scientific) platform.<sup>3</sup> A total of 100 European American (EA), 100 African American (AA), 100 South West Hispanic (SWHIS) and 100 East Asian American (EAA) population samples were selected and genotyped using the 74-plex MPS forensic assay. Allele frequencies were further generated to create allele frequency databases specific for each population group tested. In addition, a set of 10 unknown testing-samples representative of each population group was genotyped and related biogeographic ancestry inferred by calculating the random match probability (RMP) in the four corresponding American populations. The RMP calculated for the full set of samples was found remarkably higher for all those populations where individuals self-identified as such. Moreover, likelihood ratio (LR) was also calculated by dividing the highest RMP value obtained for the four tested populations by the second highest RMP value. The resulting LR value provides an indication of how much more likely it is to observe the MH profile of interest if it originated from an individual from the population at the numerator than if it originated from an individual from the population at the denominator. The biogeographic ancestry of the full set of testing-samples was correctly predicted using the allele frequencies generated from the four available in-house genotyped population groups. The level of heterozygosity of each MH locus was also calculated along with the power of exclusion (PE) to determine how good the MH markers are at discriminating individuals and excluding a random person as a possible contributor of an allele at a given locus, respectively. The heterozygosity ranged from 0.40 to 0.85 and the PE from 0.10 to 0.50. Finally, STRUCTURE-based iterative Bayesian clustering software was used for the analysis of population structure and to further confirm the origin of unknown testing-population samples.

These preliminary results suggest that the novel MPS 74-plex MH assay is an effective forensic DNA analysis tool, which provides enhanced biogeographic ancestry inference capabilities while supplementing the accuracy of existing ancestry prediction tools.

#### Reference(s):

1. Pakstis AJ, Fang R, Furtado MR, Kidd JR, Kidd KK. Mini-haplotypes as lineage informative SNPs and ancestry inference SNPs. *European Journal of Human Genetics* (2012) 20(11): 1148-1154.
2. Kidd KK, Speed WC, Pakstis AJ, Podini DS, Lagacé R, Chang J, Wootton S, Haigh E, Soundararajan U. Evaluating 130 microhaplotypes across a global set of 83 populations. *Forensic Science International: Genetics* (2017) 29:29-37.
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#### Microhaplotypes, MPS, Ancestry Inference



### **B46 Utilizing DNA Profiles Generated From Human Nail Clippings as a Means of Personal Identification**

*Jonah W.P. Stone, BS\*, Indianapolis, IN 46241; Haley Rock, BS\*, Indianapolis, IN 46227; Rachel M. Kreher, BS\*, University of Indianapolis Biology Department, Indianapolis, IN 46227; Krista E. Latham, PhD, University of Indianapolis, Indianapolis, IN 46227; Cynthia Cale, MS, Strand Diagnostics, Indianapolis, IN 46241; Gay L. Bush, PhD, Strand Diagnostics, Indianapolis, IN 46241*

**Learning Overview:** The goal of this presentation is to discuss the modified DNA extraction technique utilized in obtaining nuclear DNA from keratinized cells and how this material can be utilized for identification purposes. Following this presentation, attendees will better understand how keratinized nail material from both living and deceased persons can provide an informative DNA profile and serve as a viable alternative to the utilization of soft tissue or bone for forensic identification purposes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to a growing body of knowledge regarding alternative and often underutilized source material for the generation of informative DNA profiles for personal identification.

Common materials used for genetic studies or during the course of medicolegal investigations include saliva, blood, and bone. The body fluids should be stored in a refrigerator or freezer until DNA purification takes place, and bone potentially requires a large amount of storage space. Nail clippings have the potential to serve as a suitable source material for the generation of DNA profiles in situations where unidentified human skeletal remains are discovered or when tissue samples must be stored for later genetic analyses. If nail material is present with unidentified skeletal remains, the generation of a DNA profile from the nails would prevent the destruction of bone during the DNA purification process. In situations where tissues must be stored for extended periods of time, the clippings can be stored in small, sterile microtubes. The ability to store nail clippings, rather than vials of body fluids or sections of bone, may be useful when biological material is archived for unidentified remains cases to await technological advances or case leads.

For this study, toenails were collected from ten living and six deceased individuals to test the hypothesis that full DNA profiles can be generated from this biological source material. DNA was extracted from the keratinized cells using the QIAGEN QIAamp DNA Mini Kit with modifications to the manufacturer's instructions to allow for an extended dissolution process. The samples were then amplified with the GlobalFiler™ PCR Amplification Kit and analyzed on an AB 3130xl genetic analyzer. Comparisons were made between the profiles generated from the nail clippings and the reference profiles obtained from buccal swabs.

The DNA profiles generated from the nail clippings were single source profiles consistent with the reference samples. These results illustrate the potential for nail clippings to be utilized for the generation of DNA profiles used for personal identification purposes. The ability to utilize nail clippings for genetic identification purposes could reduce the need to store potentially biohazardous body fluids for later DNA testing or prevent the destruction of bone for genetic analysis.

#### **DNA Analysis, DNA Identification, Toenail Clippings**



### B47 Dual Separation of DNA and Peptides From Semen

Ashleigh K. Matzoll\*, University of California, Davis, Davis, CA 95616; Victoria L. Montgomery, BSc, Davis, CA 95616; Zachary C. Goecker, MPS, Visalia, CA; Glendon Parker, PhD, University of California, Davis, Davis, CA 95616

**Learning Overview:** After attending this presentation, attendees will be aware of the possibility of using both DNA and proteomics in the analysis of sexual assault kits by separating DNA from trypsin-digested peptides with filtration.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a potential new tool in the analysis of Sexual Assault Kits (SAKs) if a proteomic compatible SAK workflow is shown to be feasible.

Sexual assault is a significant issue in the United States. Many sexual assault kits collected do not contain quality autosomal DNA profiles sufficient for Combined DNA Index System (CODIS) submission. If genetically variant peptides (GVPs) are found in semen, the information inferred from them in the form of autosomal single nucleotide polymorphisms (SNPs) could be used in conjunction with short tandem repeats on the Y-chromosome (Y-STR) and/or partial autosomal short tandem repeat (STR) profiles incompatible with CODIS to increase the power of discrimination and therefore help identify the perpetrator of a sexual assault. For this process to be useful, the limited amount of sample provided in SAKs needs to be tested for both DNA and peptides, meaning DNA and peptides must be separated from the original sample, instead of sacrificing one for the other, and then analyzed with dedicated workflows for STR-typing (DNA) and tandem mass spectrometry (peptides). Selection of a compatible reagent is imperative, as reagents for DNA and peptide processing may not be compatible with other workflows. One potential approach is to exploit the size difference of digested peptides and intact DNA. For example, mitochondrial DNA has a molecular weight of approximately 10,000,000 daltons, while peptides have a molecular weight of >2,000 daltons. When a peptide mixture is digested with trypsin and then filtered through a molecular weight size-selective filter, DNA can be separated from peptides.<sup>1</sup> Trypsin was used because of its well-defined specificity. In this study, DNA and peptides were separated in one workflow and then mitochondrial DNA was quantified. Tandem mass spectrometry analysis of the peptides determined that a single digestion was sufficient to separate DNA and peptides in semen samples with the fractions suitable for subsequent analyses.

Semen samples from three individuals were incubated in 20  $\mu$ l of digestion buffer (50mM ammonium bicarbonate, 30 mM dithiothreitol, and dH<sub>2</sub>O) at 56°C for 20 minutes. Incubation continued at 37°C for 3 hours after adding 20  $\mu$ g Sequencing Grade Modified Trypsin (Thermo Pierce) and 0.01% (w/w) ProteaseMAX™ (Promega). Digests were filtered using Amicon® membrane units (Millipore); 100K MWCO. The flow-through (peptide fraction) was incubated with 60 mM iodoacetamide for 60 minutes in the dark at room temperature. For the DNA fraction, 20  $\mu$ L of dH<sub>2</sub>O were added, the membrane unit inverted, and DNA recovered by spinning 3 minutes at 1000 rcf. The final volume was brought up to 200  $\mu$ L with buffer and dH<sub>2</sub>O. Mitochondrial DNA measurements were relative and not conducted using a known standard concentration. Results showed that 22%  $\pm$  31% (average  $\pm$  SD) of DNA was retained and 0.4%  $\pm$  0.2% (average  $\pm$  SD) of the DNA flowed through. Most peptides flowed through, with 25%  $\pm$  0.2% (average  $\pm$  SD) of the peptides retained. Peptides in the filtrate exceeded the peptides found in the original samples, likely due to an inhibitor in the original samples that suppressed fluorescence, but which was removed during processing. In conclusion, the hypothesis that filters can separate out proteomic information from DNA information is viable. It is also of note that very little DNA was able to flow through the filters. However, sample loss is an issue; it is suspected that DNA was embedded in the membrane. Also, better consistency in readings is needed. Current work to resolve these issues include utilizing different membranes, adding salmon sperm DNA for less nonspecific binding, and performing a wash while the filter is upside down to extract embedded DNA. Current work also quantifies DNA using a TaqMan™ assay (Thermo Pierce) along with a standard rather than measuring relative mitochondrial DNA quantities.

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#### Filtration, Sexual Assault Kits, Proteomics



### B48 Recovery of Touch DNA: A Comparison of Four Collection Methods on Various Substrates

Rachel B. Gilmore\*, University of New Haven, West Haven, CT 06516; Emily Neverett, University of New Haven, West Haven, CT 06516; Claire Glynn, PhD, Forensic Science Department, West Haven, CT 06516

**Learning Overview:** The goal of this presentation is to inform attendees of a variety of touch DNA collection methods used globally and their suitability on porous and non-porous substrates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comprehensive comparative analysis of different touch DNA collection methods, with the intention of applying these methods to investigations of forced child labor.

It is well established that when a person comes in contact with an item, epithelial skin cells are transferred from person to surface in varying amounts. Therefore, it can be suggested that victims of forced child labor inadvertently shed their epithelial skin cells onto the items they are manufacturing. These cells can then be recovered from protected interior surfaces where only the person manufacturing the item would have touched. DNA isolated from shed cells is commonly known as touch DNA. Donor age estimation of touch DNA samples is currently being researched using DNA methylation analysis and shows great promise. It is crucial to choose a collection method that optimizes the recovery of as many cells as possible. There are several methods currently employed for touch DNA collection within accredited crime laboratories, including the wet/dry double swab method and the mini-taping method. However, there is no globally accepted standard for recovery from different substrates. An extensive search of published literature revealed the wet/dry double swab method, the sodium dodecyl sulfate (SDS) swab method, and the mini-taping method to produce the most consistently high yields of touch DNA. More recently, a novel gel film was suggested as an ideal method for touch DNA collection, with the added benefit of visualizing the cells microscopically on the gel surface prior to extraction. The aim of this research was to investigate the wet/dry double swab, SDS swab, mini-tape, and gel film methods on a variety of substrates selected to be representative of products manufactured by child laborers.

Following ethical approval from the Institutional Review Board (IRB), with informed written consent, one volunteer was selected to deposit touch DNA on all samples to ensure consistency. Eight substrates were chosen: cotton, denim, felt, polyester, plastic, ceramic tile, wood, and cardboard. For the fabric samples, to mimic the manufacturing process, the volunteer sewed a double seam on each sample using a sewing machine, thus trapping the volunteer's epithelial skin cells in the seams. For the other surfaces, flat 4" x 5" sections were rubbed by the volunteer's hand 5 times with approximately the same force applied each time. All samples were performed in triplicate and included a blank control, thereby resulting in 128 samples. Following deposition, each sample was collected using the four collection methods: wet/dry double swabbing, SDS swabbing (2% SDS solution), mini-taping (Scenesafe FAST™ Pack), and gel film (Gel-Pak®). Collection method consumables were sterilized in a UV Spectrolinker prior to use. After collection, the QIAamp DNA Investigator kit (Qiagen®) was used to extract DNA from samples, following the manufacturer's protocol. All samples were eluted in a final volume of 50 µL. All extracts were stored at -20°C until needed for quantitation. Quantitation was performed using the Qubit 3.0 Fluorometer (ThermoFisher Scientific) using the double stranded (ds) DNA High Sensitivity (HS) assay kit. A representative set of samples was chosen for full DNA profiling to compare to a known reference sample of the volunteer to ensure no contamination occurred.

Varying DNA yields were obtained from all surfaces with each collection method. The wet/dry double swab method yields ranged from 0-2.68 ng/µL. The SDS swab method yields ranged from 0-0.134 ng/µL. The mini-taping yields ranged from 0-.188 ng/µL. The gel film yields ranged from 0-.180 ng/µL. On the fabric samples, the mini-tapes appeared to produce the most consistently high yields of DNA. On the other surfaces, the wet/dry double swab method appeared to produce the most consistently high yields of DNA. The yields obtained with these two methods for the various substrates are sufficient for downstream processing, including DNA profiling and methylation analysis.

The results of this study provide a valuable contribution to the forensic science industry by highlighting optimal touch DNA collection methods for particular surfaces. Additionally, this research contributes to the ongoing efforts for age-estimation of touch DNA samples to combat forced child labor.

#### Touch DNA, Epithelial Skin Cells, Forced Child Labor



### **B49 A Comparison of Two Commercial Quantitative Polymerase Chain Reaction (qPCR) DNA Quantitation Kits for Prioritizing Forensic Samples for Downstream Genetic Analysis**

*Megan M. Foley, MSFS\*, The Center for Forensic Science Research and Education, Willow Grove, PA 19090; Catherine O. Brown, MSFS, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Heather E. McKiernan, MSFS, Center for Forensic Science Research and Education, Willow Grove, PA 19090*

**Learning Overview:** After attending this presentation, attendees will become familiar with two commercial quantitative Real Time PCR kits (qPCR), Applied Biosystems™ Quantifiler® Trio and Qiagen's Investigator® Quantiplex Pro, and their ability to accurately predict DNA quantity and quality prior to short tandem repeat (STR) analysis. Attendees will also learn how to best apply generated quantitative and qualitative results from these commercial assays to establish sample prioritization workflows, reducing cost and time associated with STR amplification and analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the strengths of two commercially available quantitative real time PCR kits supplied by different manufacturers. Both kits assess not only DNA quantity, but quality, enabling prediction of successful STR typing success allowing for optimized genetic workflows. Furthermore, this presentation will illustrate how this information can be applied for screening procedures such as Y-screening approaches for sexual assault samples.

The quantification step is a vital part of the STR profiling process that is not only required but can also be utilized to establish appropriate sample workflows, maximizing the amount of information that can be generated from a sample. Manufacturers in the field continuously optimize their products, such as quantification kits, to reduce processing time and increase reliability of the information generated. Investigator® QuantiPlex Pro (Qiagen) and Quantifiler™ Trio (Applied Biosystems) are two examples. Each assay quantifies four targets, including a large and small human autosomal target that can be used to evaluate the quality of the sample, a male target to evaluate whether the sample is appropriate for STR or Y-STR analysis, and an internal positive PCR control that can be used to identify the presence of inhibitors.

Due to the increase in the submission of evidence to be processed for DNA as well as the complexity of samples submitted, laboratories continue to look to optimize their workflows, striving for efficient but accurate results. To this end, many laboratories have adopted screening procedure for sample prioritization. Quantitation cut-off limit, male:female ratio, and information regarding the quality of a sample can all be used to prioritize samples for downstream analysis. When using quantitative information for workflow decisions, accurate and reliable results are necessary to ensure greatest success for STR genotyping analysis.

In this study, both Investigator® QuantiPlex Pro and Quantifiler Trio™ performance was assessed applying both full-scale and half-scale reactions. As both qPCR assays generated similar results regardless of the volume of the reaction scale, the remaining studies were performed using half-scale reaction volumes. A male and female dilution series of human genomic DNA were prepared using purchased cell lines. Low template samples over a range of male to female ratios were chosen to assess the correlation of low or undetermined quantitative values with the success of generating STR and YSTR profiles. Concentrations below 100 pg were amplified using Applied Biosystem® GlobalFiler Amplification Kit and YFiler® Plus Amplification Kit. The correlation between quantitation value and quantity of STR profile generated was used to determine appropriate decision cut-off points for when to stop a sample at quantitation or continue to amplification. The male:female ratio generated through quantitation was also compared to the STR profile generated in both GlobalFiler and YFiler® Plus. This correlation can be applied to Y-Screening workflows for the analysis of sexual assault samples. The correlation between quality results generated from the IPC and calculated degradation index was also compared to the quality of STR profile generated.

Based on the quantitation values obtained and the subsequent DNA profiles generated, both qPCR kits evaluated in this study performed similarly in terms of accuracy and reliability of the information generated. By implementing either kit with a quantitation-based screening workflow, a laboratory can greatly decrease the amount of time and money spent on performing DNA analysis on samples that may not generate a useful DNA profile.

#### **Quantifiler® Trio, Investigator® QuantiPlex™ Pro, Y-Screen**



### B50 MicroFLOQ®: Swabs and Direct Amplification of Trace DNA Samples

Dana M. Yenke, BS\*, The George Washington University, Washington, DC 20007; Daniele S. Podini, PhD, The George Washington University, Department of Forensic Science, Washington, DC 20007

**Learning Overview:** After attending this presentation, attendees will understand the utility of Copan's microFLOQ® swabs coupled with direct amplification for the recovery of DNA from challenging forensic samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an alternative method for recovering DNA from challenging surfaces, such as copper, by using direct amplification from microFLOQ® swabs, thereby increasing the potential yield of DNA from swabs.

Recent literature has evaluated the use of different types of swabs in terms of DNA recovery efficiency.<sup>1</sup> In comparison to traditional cotton swabs commonly used to collect biological samples from crime scenes, the use of nylon flocked swabs can increase the overall strength of a profile.<sup>1</sup> While traditional cotton swabs have fibers that circle inward towards the swab stick, nylon flocked swabs have fibers that protrude away from the swab stick.<sup>2</sup> This design allows for DNA to remain near the surface of the swab. With the DNA on the outer surface of the swab, the recovery of DNA from the swab is increased.<sup>2</sup> This makes nylon flocked swabs ideal for direct amplification, as tested by Templeton et al. microFLOQ™ swabs (Copan, Brescia, Italy) are miniature versions of nylon flocked swabs with a ~1.2mm swab head designed to easily break into polymerase chain reaction (PCR) tubes for direct amplification. Because of the demonstrated use of nylon flocked swabs for efficient DNA recovery, the goal of this study was to determine the suitability of microFLOQ™ swabs for the recovery and preservation of DNA from copper substrates.

Copper surfaces, or copper alloy surfaces such as brass used for cartridge cases, prove to be a challenge to DNA recovery because of the presence of touch DNA coupled to the degradation effects of copper on DNA.<sup>3</sup> This combination of low-level, degraded DNA often results in partial profiles, if a profile is even obtained, and indistinguishable mixtures. With the potential for loss of DNA at the extraction and quantitation steps in the DNA processing workflow, direct amplification offers a solution for unnecessary DNA loss.

MicroFLOQ™ swabs were used to swab a variety of surfaces to compare the yield of DNA from different substrates. Sterile Falcon tubes were used for a plastic control substrate; metal substrates used included cleaned, unfired bullets and cartridge casings made of aluminum, nickel, brass, and copper, in addition to cleaned copper pipes. All samples were rolled between the hands of individuals to deposit DNA. Dry-swabbing and wet-swabbing were both tested as methods to recover the DNA from samples. For wet-swabbing, bovine serum albumin (BSA) (at concentrations of 1mg/mL and 50mg/mL), sterile water, and low TE buffer were added to the microFLOQ™ swabs prior to swabbing. The swabs were processed for direct PCR amplification followed by capillary electrophoresis separation using the Identifiler® Plus amplification kit (ThermoFisher Scientific, Waltham, MA). Analysis of the profiles was followed by determining the number of alleles present in the profile compared to the expected number of alleles and the average peak height (in relative fluorescent units) per dye channel.

The results of this study indicate microFLOQ™ swabs coupled to direct amplification for short tandem repeat (STR) analysis provides a simplified approach to the recovery of DNA from metal surfaces, including copper. Partial DNA profiles were obtained from copper substrates using microFLOQ™ swabs, although the profiles recovered from copper substrates were considerably weaker compared to the profiles obtained from plastic substrates.

Additional testing was performed to determine if the degradation effects of copper can be transferred to other substrates. This was simulated by having individuals handle copper pipes immediately prior to handling plastic Falcon tubes. For comparison, a plastic tube was handled prior to handling a second plastic Falcon tube. The difference in number of alleles obtained between the subsequently handled plastic tubes were compared. While the number alleles obtained between the copper and the first-handled control plastic were significantly different, the number of alleles obtained between the second-handled plastic tubes were comparable, indicating that the degradation effects of copper are not transferred to secondary surfaces.

These results support the use of microFLOQ™ swabs coupled to direct amplification as a viable option for minimal sample handling to obtain DNA profiles from copper surfaces.

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#### Direct Amplification, Flocked Swabs, Copper Degradation

## B51 Optimal Small-Molecular Reference RNA for MicroRNA-Based Body Fluid Identification

Shuntaro Fujimoto, MS\*, Kyoto University Graduate School of Medicine, Kyoto-shi, Kyoto 606-8501, JAPAN; Sho Manabe, PhD, Kyoto University Graduate School of Medicine, Kyoto 606-8501, JAPAN; Chie Morimoto, MS, Kyoto University Graduate School of Medicine, Kyoto 606-8501, JAPAN; Munetaka Ozeki, PhD, Kyoto University Graduate School of Medicine, Kyoto 606-8501, JAPAN; Yuya Hamano, PhD, Kyoto University Graduate School of Medicine, Kyoto 606-8501, JAPAN; Keiji Tamaki, MD, PhD, Kyoto University Graduate School of Medicine, Kyoto 606-8501, JAPAN

**Learning Overview:** After attending this presentation, attendees will better understand the importance of small-molecular reference RNA for forensically relevant Body Fluid Identification (BFID) based on quantitative PCR (qPCR) and the approach for determining the optimal reference RNA.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying the optimal small-molecular reference RNA using the aggregate rank approach based on quantitative values and the expression consistency, and the development of miRNA-based BFID systems using the optimal small-molecular reference RNA identified.

Small-molecular RNAs such as microRNAs (miRNAs) are expressed in a cell-specific manner and resistant to environmental factors, and miRNA-based BFID systems have therefore attracted research attention worldwide. In qPCR-based BFID systems, the expression of the RNA is evaluated as  $\Delta C_q$ -value calculated by means of robust normalization performed using reference RNAs. The selected reference RNA must be expressed consistently among all samples and all individuals, and the copy number of the RNA should be high because the RNAs that are expressed at high levels tend to exhibit more stable expression in qPCR-based detections. In addition, normalization with multiple reference RNAs can be a reasonable solution in certain cases. The aim of this study is to identify the optimal reference RNA and the combination of reference RNAs in forensically relevant body fluids.

From five volunteers, each who provided their written informed consent, four forensically relevant body fluid samples were collected: venous blood, saliva, semen, and vaginal secretions. First, 11 candidate reference RNAs were quantified by qPCR using the miRCURY LNA<sup>TM</sup> Universal RT miRNA PCR System (Exiqon), which was a SYBR Green dye-based method to increase the specificity of binding to target sequences using locked nucleic acid technology in amplification primers. All qPCR results were obtained under compliance with the essential information provided in the MIQE guidelines.<sup>1</sup> Then, consistency of expression of candidate RNAs in body fluids was evaluated by NormFinder and BestKeeper, and last the optimal reference RNA and the combination of the multiple RNAs were determined based on each rank and index output from two tools using the RankAggreg package of R.<sup>2-4</sup> This study was approved by the ethics committee of the Graduate School of Medicine of Kyoto University.

The aggregate rank approach showed that 5S-rRNA was the best, it was followed by miR-103a-5p, miR-484, RNU1A1 and miR-92a-3p, that the best pair was the combination of 5S-rRNA and miR-92a-3p followed by 5S-rRNA and miR-484, and 5S-rRNA and miR-103a-5p, and that the best trio was the combination of 5S-rRNA, miR-484 and miR-92a-3p followed by 5S-rRNA, miR-103a-5p and miR-484, and 5S-rRNA, miR-103a-5p and miR-92a-3p. The present study concluded that 5S-rRNA was the optimal reference RNA for the normalization of miRNA expression in forensically relevant body fluids and 5S-rRNA combined with miR-92a-3p and/or miR-484 enhanced the normalization quality. Our findings enable us to perform stringent normalization of the expression of body fluid-specific RNAs, and thus can contribute to the development of small RNA-based BFID systems.

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### MicroRNA, Body Fluid Identification, Quantitative PCR



### B52 The Forensic Value of Electrospun Nanofiber Mesh for Sexual Assault Samples

Juliette Smith\*, Edmond, OK; James P. Creecy, PhD, University of Central Oklahoma, Edmond, OK 73034

**Learning Overview:** After attending this presentation, attendees will understand the forensic value of electrospun nanofiber mesh in sexual assault casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining the potentially groundbreaking idea that electrospun nanofiber mesh could aid in the separation of sperm cells from female epithelial cells in sexual assault samples.

According to the Bureau of Justice Statistics of the U.S. Department of Justice, approximately 323,450 rapes/sexual assaults took place in 2016.<sup>1</sup> The current methodology for separating the DNA of the perpetrator from that of the victim is the same methodology originally described in 1985. It is the current standard for sexual assault cases. However, that is not to say that it is without weaknesses. It is labor-intensive; some sample is lost during the washes and carryover occurs between the male and female fractions.<sup>2</sup>

Electrospinning is a technique that results in the production of nanofibers of different materials in various fibrous formations. The porosity of the resulting nanofiber formations is controllable. The head of a human sperm cell has a cross-section of approximately 5  $\mu\text{m}$  by 3  $\mu\text{m}$ . The vagina is lined with a 0.2 mm thick layer of squamous epithelial cells that is composed of four layers with the following epithelial cell diameter dimensions: superficial—50-60  $\mu\text{m}$ , intermediate—30-50  $\mu\text{m}$ , parabasal—approximately 20  $\mu\text{m}$ , and basal—12-14  $\mu\text{m}$ .<sup>3</sup> Based on these dimensions of both sperm and epithelial cells, electrospun nanofiber meshes could be designed with a specific porosity for discrimination between sperm cells and epithelial cells in a mixed sample based on size. It would allow the transport of sperm cells across the mesh, while simultaneously acting as a barrier to keep the epithelial cells from permeating.

The goal of this research is to accomplish the development of an electrospun nanofiber mesh with proper dimensions and adequate strength to separate sperm cells from epithelial cells based on size without lysing the cells in the process. Accomplishing a successful mechanism for doing so would result in cleaner differential extraction results, and therefore, cleaner profiles for a DNA analyst to interpret. This would eliminate the subjectivity associated with current analysis and interpretation due to the carryover between the male and female fractions. This would ultimately give electrospun nanofiber meshes relevance within the field of forensic science with the goal of assisting in determining the perpetrators in the hundreds of thousands of sexual assaults that occur annually within the United States.

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**Nanofiber Mesh, Sexual Assault, DNA Analysis**



### B53 DNA Methylation-Based Assay for the Identification of Smoking Status

Hussain J.H. Alghanim, MS\*, Florida International University, Miami, FL 33173; Bruce R. McCord, PhD, Florida International University, Miami, FL 33199; Wensong Wu, PhD, Florida International University, Miami, FL 33199

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**Learning Overview:** The goal of this presentation is to demonstrate an effective method to determine the smoking status of individuals based on DNA samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing a new method using a pyrosequencing-based technique to predict smoking status of a suspect from the blood or saliva samples recovered from the crime scene. This presentation will illustrate a 4-CpGs assay used to differentiate between current, former, or never smokers.

DNA methylation signatures of candidate sites have been shown to serve as useful biomarkers for various traits. Interest in such applications has resulted in several genome wide association studies using large scale epigenetic arrays. However, because DNA methylation analysis is mainly performed by array studies which require laborious bioinformatic analysis, applying DNA methylation is still difficult in the clinical and forensic regimes due to the complexity of the instrumentation and the need for relatively large sample quantities. The authors decided to examine and sequence some of these locations using bisulfite modified PCR followed by pyrosequencing. Pyrosequencing is a technique that can measure the relative methylation level at each CpG site at high accuracy (the p indicates that C and G are connected by a phosphodiester bond).

In this study, pyrosequencing techniques were utilized to identify CpG sites indicative of tobacco smoking by investigating DNA sequences surrounding ten frequently reported smoking-related CpGs at six genetic loci. The authors examined a total of 88 CpG sites located at the six genetic loci to check their association with tobacco smoking. Blood and saliva (buccal swab) samples (n=161 each) were collected from volunteers and were categorized into three groups based on their self-reported smoking history: never smokers, former smokers, and current smokers. The entire samples were randomly divided into three sets: discovery, training, and validation. DNA samples were extracted, and bisulfite modified to convert the unmethylated cytosines to uracil while maintaining the methylated ones as cytosine. Next, the DNA was PCR amplified, and the methylation level at each CpG site was quantified by pyrosequencing.

In this study, the authors identified novel smoking-specific CpG sites in various genes. Overall, 15 CpGs in blood and 10 CpGs in saliva showed a significant decrease in methylation level with current smokers. Second, a quick and inexpensive assay was developed consisting of four consecutive CpG sites at Aryl-Hydrocarbon Receptor Repressor (*AHRR*). This assay can be used to measure the DNA methylation patterns in blood and saliva. Using a multinomial regression model, the assay was utilized to differentiate the smoking status of various individuals.

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#### Tobacco Smoking, DNA Methylation, Pyrosequencing



### **B54 Fast Analysis Workflow With No Sample Preparation for Forensic Applications Using Open Probe Fast Gas Chromatography/Mass Spectrometry (GC/MS)**

*Luis A. Cuadra-Rodriguez, BS\*, Santa Clara, CA 95051; Bjorn Flatt, PhD, Agilent Technologies, Santa Clara, CA 95051*

**Learning Overview:** After attending this presentation, attendees will understand how the Open Probe Fast GC/MS system works in the forensic drug analysis workflow for compound identification with no sample preparation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating an alternate technique for fast analysis for a forensic application relating to the positive identification of compounds in a variety of samples ranging from liquids to solids. All of this is achieved by using gas chromatography/mass spectrometry system (GC/MS) coupled with an Open Probe Fast GC device and the routinely used National Institute of Standards and Technology (NIST) library searches.

The need for fast analysis for the identification of compounds in a variety of samples have been steadily increasing over the last one to two decades, especially for seized drugs. Positive identification of drugs and other chemicals in bulk samples is critical during screening in crime laboratories. Conventional drug analysis often requires sample preparation that includes dissolution, dilution, and several reagent-based assays to classify the type of drugs followed by gas chromatography-mass spectrometry (GC/MS) analysis and/or other techniques for confirmation. A simple and fast analysis workflow that does not require sample preparation is demonstrated with Open Probe Fast GC/MS. This system was equipped with 1.5m x 0.25mm (0.1 $\mu$ m 100% dimethylpolysiloxane film) and 0.8m x 0.18mm (0.18 $\mu$ m 100% dimethylpolysiloxane film) restrictor columns using a ~400°C/min temperature ramp that allowed for chromatographic separation in under 1 minute. Individual samples (liquid, solid, powder) were touched with a glass probe and introduced into the Open Probe FastGC/MS system (with single quadrupole or SQ) for 3-6 seconds for vaporization prior to data acquisition. Correct compound identification of drugs in liquids and solids is achieved through NIST library search when using a single quadrupole mass spectrometer at unit resolution. A variety of drug samples were analyzed, including drug mixtures (40-75 ng/ $\mu$ L) in solvent, tablets (whole oxycodone, pulverized hydrocodone-acetaminophen, diphenhydramine, sildenafil) and seized drugs from criminal cases including: black tar heroin, magic mushrooms and a marijuana edible. The fast-chromatographic separation, direct sample introduction and short acquisition (<1 minute) allowed for rapid and high throughput analysis of different types of samples - liquids, solids and powder each of which contained drugs. Drug compounds in a solution containing caffeine, methadone, codeine, 6-monoacetylmorphine (6-MAM) and morphine were all identified with match scores greater than 800 when using a SQ mass spectrometer. Similarly, positive identification of over-the-counter and prescription tablets was achieved without sample preparation with resulting library matches greater than 850. For a pulverized tablet (5 mg hydrocodone-300 mg acetaminophen), acetaminophen and hydrocodone were also confidently identified, although hydrocodone accounted for only 1% of the tablet mass. Additionally, the relative content (1.6%) of hydrocodone-to-acetaminophen was accurately determined by the peak areas ratios of the compounds. Analysis of real case samples resulted in the correct identification of the main drug as well as secondary components without performing any sample preparation. For example, black tar heroin analysis showed diacetylmorphine, noscapine and papaverine and a marijuana edible showed dronabinol, cannabichromene, cholesterol and squalene. The fast analysis did not require sample preparation and allowed for a simple workflow to expedite screening in a forensics application and included the following steps: 1) run blank, 2) run sample, (3) run blank and (4) run standard for confirmation. This analysis workflow resulted in overall screening and confirmation (when running a standard) of < 5 minutes for target analysis of drugs. It can also be expanded to other fields that require fast screening and identification such as homeland security and organic synthesis.

#### **Drug Analysis, Open Probe, Fast Analysis**





### **B55 The Fusion of Electrochemical and Spectrochemical Data for the Detection of Organic and Inorganic Gunshot Residues (GSR)**

*Luis E. Arroyo, PhD\**, Department of Forensic and Investigative Sciences, Morgantown, WV 26506-6121; *Korina Menking-Hoggatt, MSc*, Morgantown, WV 26501; *Tatiana Trejos, PhD*, West Virginia University, Morgantown, WV 26506

**Learning Overview:** The goal of this presentation is to demonstrate the combined use of electroanalytical and spectrochemical methods for the detection of firearm discharge residues.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a first-generation screening method that allows on-site analysis with fast detection and high accuracy. The data fusion of the organic (OGSR) and inorganic residues (IGSR) involves both selectivity and sensitivity of the analysis.

Current gunshot residue (GSR) confirmatory methods are either costly and time-consuming (SEM-EDS) or destructive of the sample (LC/MS). The incorporation of a fast and reliable screening test could streamline the current turnaround time in firearm investigations, but such methods are not currently available. In this study, electrochemical (Square Wave Anodic Stripping Voltammetry) and laser-based spectroscopic methods (LIBS) are proposed as screening assays that are quick, selective, and effective. These methods offer superior information by simultaneous detection of organic and inorganic gunshot residues, including a substantial number of elements used in modern ammunition. Moreover, the selected analytical scheme permits subsequent confirmatory analysis (SEM-EDS) on the same sample. LIBS is a rapid chemical analysis technique that uses a pulsed laser for direct qualitative and quantitative analysis of materials with no sample preparation and minimal destruction. LIBS allows high-speed measurements (usually 30-50 seconds for multiple shot analysis) and simultaneous multi-element detection in the low ppm range. On the other hand, electrochemical sensors use electrical stimulation to induce redox reactions of the analyte at the surface of the electrode. Inorganic species (e.g., Pb, Cu, Zn, Sb) and organic species (e.g., nitroaromatics, nitroamines) are electroactive, allowing the detection at low ppb levels. Electrochemical methods offer several advantages including rapid response, low cost, excellent sensitivity, good selectivity, and potential for miniaturization.

Optimization of the LIBS and electrochemical sensors was conducted using response surface Box Behnken experimental designs. Standards were prepared by spiking 50ng to 25 ug of Pb, Ba, Sb, Cu, Ti and Zn over a surface of approximately 1cm<sup>2</sup> of SEM carbon adhesives. Moreover, in-house GSR particulate standard, characterized by SEM-EDS and ICP/MS methods, were used for the optimization process. The rapid scanning of the laser beam and the voltammetric cycling allows separation and identification of trace elements (Pb, Ba, Sb) and organic compounds (e.g., DNT, NG) in less than five minutes per sample.

From a set of 200 samples, 50 samples from shooters and 50 samples from non-shooters, for up to 4 hand specimens per individual were collected as part of the validation study. Pistols (9 mm and .22) and a revolver (.357 Magnum) were fired at indoor and outdoor shooting ranges. Metrics of performance, such as error rates (false positives and false negatives), specificity, sensitivity, and accuracy, indicate the fusion of LIBS and electrochemical data are a reliable and promising approach to advance current practice. The accuracy of classification of samples into shooter vs. non-shooters groups improved from 75% (EC), and 83% (LIBS) to 98% (EC+LIBS) when the data from both sensors was fused.

#### **Gunshot Residues (GSR), LIBS, Electrochemistry**



### **B56 The Adsorption and Persistence of Gasoline Residues on Household Materials Investigated by Inverse Gas Chromatography**

*Eric Fraunhofer\**, Buffalo, NY 14223; *Joonyeong Kim, PhD*, Buffalo State College, Buffalo, NY 14222

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**Learning Overview:** After attending this presentation, attendees will better understand macroscopic transfer and persistence of gasoline residues on solid substrates, with reporting on estimated molar adsorption enthalpies and adsorption amounts of selected hydrocarbons in gasoline residues on household materials investigated via inverse gas chromatography. In addition, distortion effect of chromatograms due to the difference adsorption affinity of individual components in gasoline to solid substrates during sampling process will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a molecular level understanding of transfer, persistence, and recovery of gasoline residues on various solid substrates in forensic fire investigations.

The transfer and persistence of ignitable liquids on human skin and various daily household materials such as clothes, shoes, carpets, and wood has attracted a lot of attention as a part of efforts to reconstruct fire investigations. Previous studies have shown that the transfer and persistence of ignitable liquids on solid substrates is affected by many factors including the compositions of ignitable liquids, temperature, air movement, and physical characteristics of solid substrates. Transfer and persistence of ignitable liquids can be viewed as the adsorption of molecules in ignitable liquids on the solid substrate. Although it is a much more complex process, adsorption is the result of molecular interactions between ignitable liquids and solid substrates under the given experimental parameters. When spiked on the solid substrate, all components in ignitable liquids will be partitioned to either a gas phase via evaporation or a solid surface via adsorption with different proportions depending on the magnitude of interactions with solid substrates.

The interactions between molecules and solid substrates mainly depend on several factors including the polarity, size, and structures of adsorbing molecules (adsorbates) and chemical compositions and structures of solid substrates (adsorbents). One of the physical quantities to measure the absolute magnitude of interactions between molecules and solid substrates is the heat of adsorption, the energy required to remove the given molecule from the surface. In addition, sorption isotherms and solubility coefficients are also useful physical quantities which represent interactions between adsorbates and solid substrates. In this respect, inverse gas chromatography is one of the techniques which can estimate molar enthalpies of sorption, sorption isotherms, and solubility coefficients between adsorbates and adsorbents.

Inverse gas chromatography is a molecular probe technique used for the characterization of surface and bulk properties of various solid materials including polymers. As the reverse of a conventional gas chromatographic experiment, inverse gas chromatography utilizes a conventional gas chromatography, with minor modifications, to measure the interactions between a pure probe (e.g. hydrocarbons) and a stationary phase (e.g., polymers) in terms of the retention time of the probe as a function of temperature. A chromatographic column (stationary phase) can be packed with the solid substrate of interest which provides potential application of inverse gas chromatography for various types of solid substrates. A small quantity of pure probe compound is injected, vaporized, and diluted in a mobile phase in a stationary phase (the column) by establishing fast equilibrium between the vapor and the stationary phase. Therefore, the retention time of the injected probe is affected by the magnitude of interactions with the stationary phase. From the measured retention times of the injected probe as a function of temperature at a constant flow-rate and column inlet pressure, various thermodynamic data as well as isotherms be estimated.

The scope of this study is the investigation of hydrocarbon adsorption on solid substrates via inverse gas chromatography for a better understanding of macroscopic transfer and persistence of gasoline residues on solid substrates. Although numerous components exist in gasoline residues, six hydrocarbons (*n*-heptane, *n*-octane, *n*-nonane, toluene, *p*-xylene, and 1,2,4-trimethylbenzene) were selected as molecular probes in this work. Columns used in inverse gas chromatography measurements were prepared with three popularly encountered household materials, cardboard, cotton fabric and carpet. Herein, estimated molar enthalpies of sorption of six hydrocarbons on three solid substrates, isotherms of these hydrocarbons on three solid substrates measured at 40°C and the solubility coefficients of these compounds on solid substrates at 40°C as defined by Henry's law are reported in the presentation.

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#### **Inverse Gas Chromatography, Gasoline Residues, Adsorption Enthalpies**

### B57 The Optimization of Superglue Fuming to Develop Fingermarks Exposed to Different Bodies of Water

Emily L. Horrocks, MSc\*, University of Portsmouth, Portsmouth, Hampshire PO1 2QQ, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will learn the most effective way to recover fingermarks from wet items using superglue.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how cyanoacrylate is not normally considered for wet items, but this research shows the potential use and effectiveness of this method, impacting evidence value of wet items.

It is important to develop techniques and methods to maximize evidence potential from a range of different environments and situations. There has been little research into the recovery of fingermarks exposed to water and how the treatment methods work. Furthermore, the application of more commonly used fingermark recovery methods has not been fully explored for wet items. The small variety of research completed in this area demonstrates the evolution of techniques and new emerging ideas that can be applied to the adverse conditions presented by an underwater crime scene.<sup>1-7</sup> There is a need to fully evaluate each method so that the application to underwater investigation can be understood.

There is much conjecture around what initiates the reaction with cyanoacrylate, allowing the marks to be visualized. Some research suggests water, while others investigated specific components of the fingermark.<sup>8,9</sup> If water were the initiating component, it would explain the belief that superglue is a poor method for wet items, even when they have been dried before fuming. It is thought that the water in a fingermark is removed during submersion in the water. Therefore, there is only the other components left in the mark and nothing to initiate the cyanoacrylate or for the glue to adhere to. However, if the carboxylic acid group is the initiator, these components survive in water and could explain the positive results submerged fingermarks have with cyanoacrylate.<sup>9</sup> Ultimately, by showing this is what the superglue adheres to, it demonstrates the application cyanoacrylate can have for items exposed to water.

During a series of experiments, testing a variety of methods, it was discovered that cyanoacrylate could efficiently recover fingermarks from knives that had been submerged in three different aquatic environments. Cyanoacrylate was in fact, the most effective method, when compared to black powder suspension, iron oxide and small particle reagent, which are recommended for wet items.<sup>10</sup> The effect each water type has on the superglue process was observed and a variety of methodologies developed to identify the best approach for each condition. This study explores the pre-treatment and individual fuming conditions needed to recover fingermarks from knives from each water type used: Sea water, harbor water and river water. The results show variation in treatments, with salt water producing the best quality fingermarks. Further studies into the levels of salt and how the salinity effects the cyanoacrylate are needed to identify why this method is so effective. Furthermore, pre-treatments such as salt washes, may improve quality of the fingermarks across the three water types by increasing the minerals in the mark, the component that, in this study, the cyanoacrylate responds best to. Ultimately, this research will improve the value and effectiveness of forensic methods for underwater investigation by providing another method for practitioners to recover fingermarks from items found in water.

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#### Superglue, Fingermarks, Underwater



### B58 Blood Decomposition Odor Profiling: An Instrumental and Field Application Tool in Forensic Detection

*Kiana L. Holbrook, BS\*, Lubbock, TX 79401; Paola A. Prada, PhD, Texas Tech University, Lubbock, TX 79416*

**Learning Overview:** The goal of this presentation is to provide better understanding of the specific Volatile Organic Compounds (VOCs) emitted from decomposing blood evaluated over specific time intervals and how the detected blood odor profile can be utilized as a marker of chemical detection effectiveness during field work applications.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by its novelty of being one of the first studies to evaluate and created a blood decomposition profile and one of the first to evaluate commonly used presumptive reagents like Bluestar® and Luminol to correlate the field response with the specific odor signature for a blood decomposition time frame.

This presentation will deliver strong scientific perspectives, implementing both chemical laboratory and field findings, regarding the characterization of blood odor volatiles as a function of decomposition time. Blood is a vital biological body fluid found in most living organisms. Blood is an important serological medium which can be tested to detect diseases, inflammation, pregnancy, infections, anemia, and other conditions like cancers or bleeding disorders. DNA and blood pattern analysis in criminal investigations is crucial in determining the victim(s) or suspect(s), the velocity, distance, weapon, and impact utilized during a crime. However, there is limited literature on the correlation between blood analysis and the victim's time of death. Therefore, this study sheds light on the odor volatile composition of a decomposing blood sample matrix as a function of time. This research further enhances optimum serological (blood) evidence decomposition detection procedures for purposes of estimating time of death based on the emitted VOCs. A novel approach to this study is that it also integrates the detection effects of presumptive reagents in response to the blood decomposition time frame. The optimal implementation of blood decomposition odor profiling and the ability of presumptive reagents to detect levels of decomposed blood impacts the forensic field by providing a valuable, highly deployable lab, and field tool for the biological detection of evidence.

There is a research gap in the evaluation of blood decomposition processes and the use of potential blood decomposition odor markers in establishing time of death ranges. Onset of coagulation in terms of blood decomposition can potentially display vital signs in determining how long the blood has been drying. Research exploiting the use of canines for cadaver detection has associated challenges regarding the age of the blood used as a training aid, and ultimately a lack of scientific foundation as to the odor signatures with respect to decomposition time. This novel research is an evaluation of how many volatile organic compounds (VOCs) are present during various stages of decomposition. This study will be the first to conduct blood decomposition odor profiles for the presence of VOCs using a GC/MS (gas chromatograph/mass spectrometer) targeting specific time windows as a function of volatile odor patterns. The purpose of this research further intends to increase the knowledge of detection windows of presumptive reagents, Bluestar and Luminol, by introducing a parallel understanding of distinctive volatile odor profiles in relation to the number of resulting false positives and the observable intensity based on apparent fluorescence. This study analyzed blood at three distinctive time frames of decomposition: fresh (0-48 hours), intermediate (49-96 hours), and late (97-168 hours) potentially revealing different VOCs specific to each phase. Instrumental evaluation utilized Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/ PDMS) coated Solid Phase Microextraction (SPME) fibers that were injected into a GC/MS system for the identification of extracted blood decomposition odor profiles at each of the three decomposition time frames. Within the three-phase, two substrates were analyzed: cotton gauze pads and metal nails (zinc/iron and copper), to determine if the amount of detected VOCs present in natural blood decomposition is affected by the substrate's composition.

The significance of this work was to show that the odor profile resulted in several volatile organic compounds of varying functional groups which could potentially suggest and support how long the victim has been dead (postmortem interval (PMI)) regarding the deposition of blood at a crime scene. It is also one of the first studies to evaluate commonly used chemical reagents such as Bluestar and Luminol and correlate the field response with the specific odor signature for that blood sample time frame. This research presents a bridge to the knowledge gap of blood odor profile composition and presumptive reagents.

#### **Blood Decomposition VOCs, Solid-Phase Microextraction, GC/MS**



### B59 Characterization and Comparison of Electrical Tape Backings by X-Ray Fluorescence (XRF)

Meghan Prusinowski\*, Morgantown, WV 26505; Andria H. Mehlretter, MSFS, FBI Laboratory, Quantico, VA 22135; Claudia Martinez, BSc, Florida International University, Miami, FL 33199; Jose R. Almirall, PhD, Florida International University, Miami, FL 33199; Tatiana Trejos, PhD, West Virginia University, Morgantown, WV 26506

**Learning Overview:** After attending this presentation, attendees will understand: (1) the importance of elemental analysis in the comparison of electrical tape backings; (2) the advantages of utilizing XRF for characterization of the backing composition; (3) the considerations that must be made for sample preparation when using XRF; and (4) a method for quantitative assessment and comparison of the spectra.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the viability of XRF for the analysis of electrical tape backings. In addition, this presentation provides statistical analysis for the spectral data that aids in the classification and comparison of samples.

Elemental analysis is one of the most informative steps in the forensic examination of electrical tapes. The detection of inorganic elements provides input regarding the chemical composition of fillers, polymers and other additives of the backings. The analysis is typically conducted by Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS), although Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) has recently shown to enhance the certainty of the determinations.<sup>1</sup> In this study, the utility of X-Ray Fluorescence (XRF) is compared to previously published SEM-EDS and LA-ICP-MS data. Three different XRF systems were used to evaluate a range of system configurations commonly available at crime laboratories. A set of 40 electrical tape backings known to originate from various sources was used to assess the inter-roll variability, discrimination and classification capabilities of the method. The improvement in discrimination ranged from 78.8% (SEM-EDS) to 81.5 to 91.0% (XRF) depending on the instrument configuration, to 84.6% (LA-ICP-MS). The characterization of elemental profiles and classification into different groups improved with superior sensitivity and selectivity of the methods. SEM-EDS detected up to 8 relevant elements, while XRF and LA-ICP-MS detected up to 14 and 29, respectively. As a result, classification capabilities and accuracy improved with the use of XRF and laser ablation methods. A set of 20 pieces of tape collected from the same roll were analyzed to evaluate the intra-roll variability. Duplicate control samples from the same tapes were used to assess inter-day and intra-day instrument variability. No false exclusions were observed in the data set, demonstrating the within sample variability and instrumental variability are relatively lower than the inter-sample variability. XRF showed to be a viable analytical tool for the forensic examination of electrical tapes, with advantages of speed of analysis, non-destruction of the tape, and high informing power.

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#### Electrical Tape, X-Ray Fluorescence, Elemental Characterization



### **B60 Differentiation of Isobaric and Isomeric Fentanyl Analogs by Gas Chromatography/Mass Spectrometry (GC/MS)**

*Jianmei Liu\*, Cayman Chemical, Ann Arbor, MI 48108; Roxanne E. Franckowski, MS, Cayman Chemical Company, Ann Arbor, MI 48108*

**Learning Overview:** After attending this presentation, attendees will understand the differentiation of isobaric and isomeric fentanyl analogs using Gas Chromatography/Mass Spectrometry (GC/MS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the GC/MS data of 60 compounds grouped into the different structural and geometric (cis-trans) isomeric forms of fentanyl analogs. A scheme for separating and identifying each of the components will also be presented.

Fentanyl, a powerful pharmaceutical grade opioid, has been used for decades to treat pain. Numerous fentanyl-type compounds began to emerge in the illicit market beginning in 2015. To date, a wide variety of fentanyl analogs, such as cyclopropyl fentanyl and 3-methylfentanyl, have been identified by forensic practitioners. Isobaric and isomeric analogs of fentanyl pose challenges for identification and differentiation of these compounds in forensic casework.

In this study, 60 of fentanyl analogs, including structural and geometric isomers, were analyzed by GC/MS. GC/MS conditions: column, Restek, Rtx-5 MS, 30 m × 0.32 mm I.D., 0.5 μm film thickness (Phase composition, Crossbond 5% diphenyl / 95% dimethyl polysiloxane, similar columns: DB-5MS); oven temperature, 100°C (1min hold) and programmed up to 300°C at a rate of 20°C/min; injection port temperature, 300°C; carrier gas, helium at the rate of 2.0mL/min; sample injection, split mode with the split ratio of 15:1; ionization, Electron Ionization (EI); electron energy, 70 eV; the transfer line temperature was 300°C, and the source temperature was 230°C. Each solution was prepared by dissolving 1mg of each component in 1mL of HPLC-grade methanol. 1.0μL injection of each 1.0mg/mL solution was analyzed.

Extracted Ion Chromatogram (EIC) function was used to select key fragments of the fentanyl analogs. Relative retention time (RRT) was used to minimize the impact of retention time variation. RRT is expressed as the ratio of retention time of a compound to the internal standard. Fentanyl was introduced as an internal standard and mixed in all samples. The combination of RRT and EIC were used to identify each component.

Five positional or geometric isomers of the fluoro substituted 3-methyl fentanyl were separated by slowing the temperature ramp to 3°C/min. Similar groups were also separated by GC/MS. Oven temperature was determined to be critical to achieve successful separation. By slowing down the rate of the oven temperature program, complete chromatographic separation and baseline resolution of more than 1.5 was achieved.

To determine if the tuning of the mass spectrometer influenced the ratio of the characteristic fragment ions various tuning types, such as the Standard Spectra Autotune (S-tune) and Low Mass tune, were examined. The effects of the alternate tuning methods on Cyclopropyl fentanyl and crotonyl fentanyl will be presented.

#### **Fentanyl, Isomer Determination, GC/MS**



### B61 An Analysis of Non-Detonable Canine Training Aids for Hexamethylene Triperoxide Diamine (HMTD)

*Alison G. Simon, PhD\*, Washington, DC 20375; Lauryn DeGreeff, PhD, U.S. Naval Research Laboratory, Washington, DC 20002*

**Learning Overview:** After attending this presentation, attendees will understand about commercial-off-the-shelf training aids of the homemade explosive HMTD for the training of explosives detection canines. Attendees will learn how the vaporous characteristics of these training aids compares to that of bulk HMTD with implications for canine training.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a method of strengthening pre-existing canine detection in forensic science and explosives through the characterization of the headspace of canine training aids used to mimic or present a non-detonable version of the extremely hazardous but field relevant explosive, Hexamethylene Triperoxide Diamine (HMTD).

Defense and security communities have typically focused on the detection of traditional explosives, but due to the increasing frequency of homemade explosives (HMEs), developing detection methods of these explosives is imperative. Explosives detection canines are trained for this purpose, protecting United States civilians and military personnel by locating or identifying the presence of an explosive device or explosive components. Canines have long been considered the gold standard of real-time standoff explosives detection, so it follows that law enforcement and military canine programs are at the forefront in developing an efficient method for detecting such materials. Explosives detection canines are typically trained to detect traditional explosives, such as TNT, RDX, PETN, dynamite, black powder, and/or smokeless powders. However, the popularity of HMEs has increased as they are manufactured from commercial ingredients and equipment that are easier to obtain than military-grade or traditional explosives. The prevalence of their various components in the environment or legitimate uses also makes them more difficult to detect by traditional methods, as does the novelty of certain mixtures.

One such commonly encountered HME is hexamethylene triperoxide diamine (HMTD), a peroxide explosive. HMTD has been recovered in the 2016 New York and New Jersey attacks as well as the 2005 London subway bombings. HMTD can be hazardous to handle due to its instability and friction sensitivity. Therefore, several companies have developed pseudo or non-detonable canine training aids to be used as a safe alternative to training on the bulk material.

Previous research shows that the headspace of HMTD is complex and varies with time, temperature, humidity, and manufacturing process. There have been no evaluations of how these variables affect canine detection of various HMTD samples that may be encountered. This study evaluated the headspace of five commercial-off-the-shelf (COTS) canine training aids for HMTD using solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC/MS) to determine how accurately they represent bulk HMTD samples. Of the six previously identified headspace components, all six were identified in the various COTS training aids analyzed, though each brand contained a different combination of these compounds. The odor profiles of the training aids varied drastically and were not consistent with bulk HMTD. Based on analytical results, it may be possible to incorporate COTS training aids as brief “snapshots” of HMTD odor, for example, when training with the live explosive is not possible due to hazards or associated costs. However, it is still recommended to train on actual substances when possible. Such evaluations have important implications for canine training and eventual success in the field.

#### **HMTD, Canine Detection, Headspace Analysis**

## B62 A Quantitative Analysis of Trace Elements in Electrical Tapes

Saeed Almheiri\*, University of Sharjah, University City, Sharjah, UNITED ARAB EMIRATES; Claudia Martinez, BSc, Florida International University, Miami, FL 33199; Jose R. Almirall, PhD, Florida International University, Miami, FL 33199

**Learning Overview:** After attending this presentation, attendees will understand the development and optimization of a new quantitative method of analysis of the elements present in electrical tapes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a method for the quantitative analysis of the elements present in plastics, specifically in electrical tapes, which can help in creating and populating databases which can lead to the use of likelihood ratios and the development of standard methods of analysis and interpretation for tape evidence.

Electrical tapes are a common and potentially important form of forensic evidence. Tapes are used for improvised explosive devices, drug packaging, illicit electrical work, and other criminal activities. For the first time, this work reports a quantitative method of analysis for the elements present in electrical tapes. In this quantitative method, a constant stream of standard solution of a mixture of elements is introduced into a spray chamber, where it is mixed with the particles resulting from the laser ablation process of a solid sample. Measurement of the solid particles' mass is achieved using a piezoelectric dust monitor (Kanomax, 3521). The solution and ablation mixture are then introduced into an inductively coupled plasma mass spectrometer (ICP-MS) where an intensity vs. time signal can be obtained for each isotope.<sup>1,2</sup>

By using a solid of known concentrations, a response factor specific to each isotope can be found and the concentrations of the elements present in an unknown solid can be calculated. Solid standards samples used for this method included: NIST SRM-610 and NIST SRM-612 glass standards and BCR-680 and ERM<sup>®</sup>-EC681m polyethylene standards.

This poster reports the theoretical principles, development, and optimization of a quantitative method of analysis of the elements presents in tapes. Laser optimization and the selection of the optimum solution concentration for the different standards are also reported.

The accuracy of the method was tested using the different solid glass and plastic standards. The bias for the NIST SRM-610 glass standard was found to be below 10% for most of the elements under study; the bias for the BCR-680 polyethylene plastic using ERM<sup>®</sup>-EC681m polyethylene plastic resulted in less than 10% for most elements under study. Tape concentrations were measured using ERM<sup>®</sup>-EC681m polyethylene as a known standard and were found to be:  $4 \pm 1$  ppm for As,  $2104 \pm 318$  ppm for Cd,  $39 \pm 6$  ppm for Cr,  $5 \pm 1$  ppm for Pb,  $37 \pm 5$  ppm for Sb, and  $1796 \pm 270$  ppm for Zn. This quantitative method can help in creating and populating databases which can lead to the use of likelihood ratios and the development of standard methods of analysis and interpretation for tape evidence. This method also has the potential to be used for different types of solids without the need to conduct acid digestions and intense sample preparation procedures.<sup>3-5</sup>

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### Quantitative Method, Electrical Tape, LA-ICP/MS





### B63 The Effect of Aqueous Foam Concentrate-380 Blast Suppression Foam on Latent Fingerprints

Kelsey Kyllonen, MA\*, *ORISE/FBI Laboratory, Quantico, VA 22135*; Jeffrey Leggitt, *FBI Laboratory, Quantico, VA 22135*; Maria A. Roberts, *Quantico, VA 22135*; Kelli Edmiston, *Quantico, VA 22135*; Keith L. Monson, PhD, *Quantico, VA 22135*

**Learning Overview:** After attending this presentation, attendees will understand the effect that Aqueous Foam Concentrate (AFC) blast suppression foam, specifically AFC-380, has on latent fingerprints that have been deposited on various substrates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing fingerprint examiners with new information regarding latent fingerprint evidence recovery and interpretation after exposure to aqueous blast suppression foam.

In addition to having blast mitigation properties, AFC-380 blast suppression foam is designed to capture aerosolized chemical, biological, and radioactive particles with over 99% efficiency during render-safe procedures of explosive devices.<sup>1</sup> The chemical composition of AFC-380 foam is similar to many commercial firefighting foams, with surfactants chemically similar to those found in liquid soaps and shampoos.<sup>2</sup> Unlike firefighting foams, AFC-380 is designed to delay water drainage from the foam matrix; consequently, dissipation of the foam takes several hours and any forensic evidence present at the scene will likely be exposed to the foam for several hours before the items can be collected safely. Exposure to aqueous environments may negatively affect preservation of fingerprint evidence and the effect of AFC-380 foam on the preservation of fingerprint evidence has not yet been investigated.<sup>3,4</sup> The purpose of this study is to assess the potential success of fingerprint evidence recovery and interpretation after exposure to AFC-380 foam.

Sebaceous fingerprints from a single donor were deposited on galvanized metal pipes, galvanized metal end caps, galvanized metal flats, PVC pipes, PVC flats, glass microscope slides, pieces of plain copy paper and cardboard, and both the adhesive and non-adhesive sides of pieces of electrical tape, packing tape, extra strength duct tape, and regular strength duct tape. The samples were divided into two identical experimental groups and a control group before being exposed to AFC-380 foam as part of a training exercise. One group of experimental items was placed next to a simulated radioactive dispersal device ("dirty bomb") and exposed to both AFC-380 foam and an explosive charge as part of the render-safe process. The second group of experimental items was placed in a large plastic tub, which was then filled with foam and placed a short distance away from the explosive device to simulate a successful render-safe procedure using non-explosive methods. Altogether, the experimental groups were exposed to the foam for approximately three hours before being removed from the foam and air-dried for 48 hours.

Non-porous items were first examined for ridge detail visually and under both ultraviolet (UV) and laser light sources. Except for the adhesive sides of the tape samples, the items were then processed using cyanoacrylate fuming and examined for ridge detail before being treated with cyanoacrylate fluorescent dye and examined again for ridge detail under both UV and laser light sources. The adhesive sides of the tape samples were processed using WetWop and examined visually. Porous items were examined visually and under both UV and laser light sources before being processed with indanedione and reexamined using UV and laser light sources.

Ridge detail was developed on 29% of the non-adhesive sides and 57% of the adhesive sides of the recovered tape samples, with no differences in print development frequency observed among the four types of tape or between the two experimental conditions. In contrast, ridge detail was not developed at any point in the processing sequence on any other experimental item. Fingerprints were developed on all control items. These results indicate that exposure to blast suppression foam may result in differential preservation of fingerprint evidence depending on the deposition substrate. Potential explanations for this are being investigated.

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#### Fingerprints, Blast Suppression Foam, CBRN



### **B64 The National Forensic Laboratory Information System (NFLIS): Monitoring Trends in Cannabis/THC, Methamphetamine, and Cocaine Drug Reports**

*DeMia P. Pressley, MS\*, Drug Enforcement Administration, Springfield, VA 22152; Liqun Wong, MS, Springfield, VA 22152; Terrence Boos, PhD, Drug Enforcement Administration, Springfield, VA 22152; BeLinda J. Weimer, MA, Research Triangle Park, NC 27709; Hope Smiley-McDonald, PhD, RTI International, Research Triangle Park, NC 27709; Katherine N. Moore, MS, Research Triangle Park, NC 27709; Jeffrey M. Ancheta, BS, Research Triangle Park, NC 27709; Neelima Kunta, BS, RTI, Research Triangle Park, NC 27709; David Heller, BS, RTI International, Research Triangle Park, NC 27709; Jeri D. Roper-Miller, PhD, RTI International, Research Triangle Park, NC 27709*

**Learning Overview:** After attending this presentation, attendees will understand the types of information provided by the DEA's National Forensic Laboratory System (NFLIS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing specific knowledge of national and regional trends for methamphetamine, cocaine, and cannabis/THC reported to the National Forensic Laboratory Information System (NFLIS).

NFLIS is a program of the Drug Enforcement Administration (DEA), Diversion Control Division. NFLIS-Drug systematically collects drug identification results and associated information from drug cases submitted to and analyzed by Federal, State, and local forensic laboratories. These laboratories analyze controlled and noncontrolled substances secured in law enforcement operations across the country, making NFLIS-Drug an important resource in monitoring illicit drug abuse and trafficking, including the diversion of legally manufactured pharmaceuticals into illegal markets. These data are used to support drug scheduling decisions and to inform drug policy and drug enforcement initiatives both nationally and in local communities around the country. Each year, NFLIS-Drug publishes an annual report on data reported to NFLIS. Part of the annual report captures national and regional estimates for the top 25 most frequently identified drugs. Over the last few years, NFLIS has shown trends with methamphetamine, cocaine, and cannabis/THC as the three most frequently identified drugs that are notable to the community.

Results of drug cases *submitted* to State and local laboratories from January 1, 2001 through December 31, 2017, which were *analyzed* by March 31, 2018, are presented for methamphetamine, cocaine, and cannabis/THC. The data presented include *all* drugs mentioned in the laboratories' reported drug items. National annual estimates and regional trends for methamphetamine, cocaine, and cannabis/THC are presented.

In the 2014 annual report, methamphetamine surpassed cocaine as the second most frequently identified drug, while in 2017, it surpassed cannabis/THC as the number one most frequently identified drug. Cocaine reports gradually increased from 2001 to 2007, then significantly decreased through 2014, followed by slight increases in reports through 2017. From 2016 to 2017, reports of cannabis/THC (from 374,712 to 344,167 reports) decreased significantly, while reports of methamphetamine (from 314,872 to 347,807 reports) and cocaine (from 214,602 to 230,436 reports) increased significantly ( $p < .05$ ). Between 2016 and 2017, cannabis/THC reports decreased significantly in the Midwest and West regions ( $p < .05$ ). Methamphetamine reports increased significantly in all regions except the West, in which reports significantly decreased. Cocaine reports increased significantly in the South and Midwest regions.

NFLIS-Drug publicly shares data that can benefit management decisions of crime laboratories through various reports throughout the year including midyear and annual reports. NFLIS-Drug provides a resource for the community to identify and respond to drugs trends.

#### **NFLIS, DEA, Drug Trends**



### B65 Identification of an Ultraviolet (UV) -Induced Promethazine Dimer

*Sarah E. Chaffman\**, Morgantown, WV 26505; *Tyler Williams*, Hanover, MA 02339; *James T. Miller*, MA, Houston Forensic Science Center, Houston, TX 77002; *J. Tyler Davidson*, MS, West Virginia University, Morgantown, WV 26505; *Glen P. Jackson*, PhD, West Virginia University, Morgantown, WV 26506-6121

**Learning Overview:** After attending this presentation, attendees will understand UV-induced degradation of promethazine in casework, which results in two chromatographic peaks with indistinguishable promethazine-like Electron Ionization (EI) mass spectra. Attendees will also learn about the application of tandem mass spectrometry for the structural characterization of the UV-induced product ion, a covalently linked promethazine dimer.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the structure, mechanism and rate of formation of a UV-induced dimer of promethazine.

**Hypothesis:** The central hypothesis is that the dimerization of the promethazine salt occurs through a process of UV exposure in the presence of chloroform. Additionally, the authors hypothesize that using a soft ionization technique such as electrospray ionization with collision-induced dissociation (ESI-CID-MS) will make it easier to detect the presence of the promethazine dimer that can be difficult to detect using traditional EI-MS. Finally, they hypothesize that with the use of tandem mass spectrometry (MS), they will be able to structurally characterize the promethazine dimer and identify structural modifications that occur due to UV exposure.

**Methods/Results:** Analyses involved dimerization of promethazine when exposed to intense short-wave UV light which was produced by the optimal crosslink setting of an XL-1500 Spectrolinker. A comparison of different UV exposure times shows that the concentration of promethazine dimer in solution increases as a function of the length of time the promethazine monomer is exposed to UV light. The process involves a 50 ppm promethazine solution poured into a covered glass petri dish, elevated by an adjustable lab stand inside the UV crosslinker and exposed to short-wave UV light. The resulting promethazine monomer and dimer were analyzed using an Agilent Technologies 7890B GC/5977A MS and a Thermo Scientific LTQ Velos Pro with HESI ionization source.

Preliminary results indicate that two different promethazine dimers are observed when using ESI: 1) a weakly associated dimer produced by clustering within the ESI source, and 2) a covalently bound dimer induced by UV exposure. These conclusions are based on the isolation and fragmentation parameters required for tandem MS. The tandem MS results indicate the weakly associated dimer fragments into monomer only, whereas the UV-induced dimer fragments into a series of ions corresponding to substituted promethazine monomer. Additionally, two chromatographic peaks are observed under traditional EI conditions. On-going reaction kinetics are focused on identifying the rate order of the conversion of promethazine monomer to dimer.

#### Drug Chemistry, Dimerization, Tandem Mass Spectrometry



### B66 Burning Down the House: The Biometric Recovery of Latent Prints and Blood in Arson Crime Scenes

*Jason A. Casper, MFS\*, Las Vegas, NV 89081; Ismail M. Sebetan, MD, PhD\*, National University, La Jolla, CA 92037-1011; Jose E. Valdez, Jr, MFS, San Diego Sheriff's Crime Lab, San Diego, CA 92123; Paul Stein, PhD\*, National University, La Jolla, CA 92037*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the feasibility and value of collecting latent print and blood trace evidence when dealing with fire and arson investigations and offer insight into the best practices for evidence recovery. It is anticipated that the findings will dispel any myths that intense heat from fire will likely destroy blood and latent print biological and physical trace evidence. The effect of exposure to heat and ability to obtain biometric evidence from “the recovery zone” should not be overlooked.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing framework for recovery of the biometric evidence after exposure to heat when investigating fire and arson casework. The presented information will improve understanding of the effect of heat on the recovery of biometric evidence.

The first objective for this investigation was to determine if the distance to high temperatures in a fire plays a significant role in obtaining evidence from the materials and containers used to initiate the fire, or arson. The second objective was to determine if the materials used to create a fire significantly impacts the collection of the related blood and latent print evidence. The third objective investigated the impact of exposure time to elevated temperature and the ability to recover the stated forensic evidence. It was felt that there would be a significant difference in ability to recover blood evidence. However, it was also felt that the difference for latent prints collected from the materials used by an arsonist would not be significant. Results will be presented based on the recovered latent print and blood evidence. Possible DNA profiles and related information will be discussed.

Several tests were conducted in this study based on varying distances to the fire heat source (propane torch) temperatures and the ability to recover latent prints and blood evidence. The exhibits were exposed to the heat source at 18 inches and 3400 degrees, 22 inches at 2720 degrees and 24 inches at 1700 degrees Fahrenheit. In each test, a control group not exposed to the heat source was included. The latent prints were detected by silver metallic dusting powder. Blood was detected using the Kastle-Meyer presumptive test with phenolphthalein reagent. Materials testing positive for blood were analyzed for DNA after collection with a moistened cotton swab.

The first research objective concluded that while fingerprints were recoverable from all the exhibits, the presumptive blood test was only negative on the aluminum substrate. This may be explained by the ability of the metallic aluminum substrate to retain heat longer than the glass or plastic. All the controls (not exposed to heat) were positive for the blood and latent print evidence. An interesting finding was that fingerprint evidence became etched into the metallic surface from the exposure to high temperatures. The second objective indicated that the recovery of evidence was impacted by the nature of the materials possibly used to contain inflammable substances used to create the fire. The third objective indicated that exposure time played a significant role in the recovery of biometric data (fingerprints, blood and DNA). Duration time did not significantly impact fingerprint recovery (100%), but recovery of blood evidence was markedly reduced (75%) dependent on the exposure time.

These results encourage practitioners to recognize the importance of not overlooking the possible recovery of latent fingerprint and blood evidence from fire-arson investigations. Exposure to elevated temperatures in our study did not impact recovery on glass, plastic and metallic substrates (containers) of latent prints. Even blood evidence was located on these objects by presumptive tests, although not on the metallic substrate, probably due to superheating preventing recovery. This raises the possibility of obtaining probative DNA profiles from some arson exhibits, linking to possible suspects.

#### Fire-Arson, Fingerprints, DNA



### B67 Trinitrotoluene (TNT) Recovery Via Microbial-Vac System (M-Vac) Collection

Jacqueline Ruchti, BS\*, IUPUI, Indianapolis, IN 46202; John V. Goodpaster, PhD, FIS Program, Indianapolis, IN 46202

**Learning Overview:** After attending this presentation, attendees will better understand the recoverability of TNT from post-blast debris after collection via the Microbial-Vac System (M-Vac).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing novel partition coefficients for TNT as well as a potential collection technique for simultaneously collecting DNA and explosive residue from post-blast debris.

Analyzing the overall construction of an explosive device, as well as the explosive used, can yield information about the bombmaker or terrorist organization and provide links to other devices. Simultaneous analysis of post-blast debris for DNA and explosive compounds could improve explosives investigations. Hence, DNA and explosive residues would need to be separated via an extraction method. Methods of liquid-liquid extraction, charcoal strip extraction, and filtration were determined for extracting 2,4,6 – trinitrotoluene (TNT) prior to traditional DNA extraction methods.

The partitioning of TNT, a common military explosive, was studied for the following four aqueous/organic solutions: water/chloroform, Lysis Buffer/chloroform, Lysis Buffer with proteinase K (incubated)/chloroform, and Butterfield's Buffer/chloroform. A liquid injection method was developed for the chloroform layer. An immersion solid-phase microextraction method was developed for the aqueous layer. Since the Log P value for TNT in an octanol/water solution is 1.6, TNT was expected to partition by approximately 40:1 between octanol and water. However, the partitioning of TNT between chloroform and Water was inconclusive. TNT partitioned by approximately 3:5 between chloroform and Lysis Buffer, 1:2 between chloroform and Lysis Buffer with proteinase K (incubated), and 35:2 between chloroform and Butterfield's Buffer.

Due to the ability of the Microbial-Vac System to collect DNA from rough and porous surfaces, the device also was tested for its effectiveness in collecting TNT. Evidence collected via the M-Vac device was analyzed by the following GC/MS application methods after extraction. Samples prepared via the chloroform partition method or charcoal strip extraction method were run via liquid injection while samples prepared using filtration method were run via liquid immersion SPME. Following the application of a 50 ppm chloroform solution of TNT to pieces of a white t-shirt, TNT was successfully detected via the chloroform partition method. To simulate samples from an explosives investigation, a backpack containing a pipe bomb filled with TNT was detonated in a secured field. However, TNT was not successfully detected in any post-blast backpack debris samples prepared via the three extraction methods. Upon performing a direct liquid extraction with other post-blast backpack debris samples, it was determined that TNT was not sufficiently present on the post-blast backpack debris samples tested. Hence, the M-Vac's efficiency for collecting TNT from post-blast debris is inconclusive.

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**TNT, Microbial-Vac System (M-Vac), GC/MS**



### B68 Split Second Decision: Is It Real or Is It Fake?

*Andrew J. Winter, MS\*, Middlesex County Prosecutor's Office, New Brunswick, NJ 08901; Peter J. Diaczuk\*, Pennsylvania State University, State College, PA 16802*

**Learning Overview:** After attending this presentation, attendees will understand: (1) the differences between an authentic firearm and a blank gun; (2) how each are designed, cycle, and discharge; and (3) the powder components of the cartridges utilized in both guns.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of blank guns and how they function and compare to authentic firearms, so that the attendee is better prepared when these guns are seen in case work.

The crime scene professional is routinely called upon to process active crime scenes where an authentic firearm designed to discharge a projectile (bullet) and other ammunition components may potentially be present at the crime scene. Less commonly seen in active case work is the crime scene where a blank gun was used which is not designed, at time of manufacturer, to discharge a projectile (bullet). The blank gun has been mistaken for an authentic firearm in police involved shootings where police officers must make a split-second decision whether to discharge their own service weapons. This split-second decision that police officers must make to shoot or not raises the question for crime scene investigators as to what the police officers saw and heard at the discharge of a blank (imitation) gun and how it compares to the discharge of an authentic firearm capable of discharging a projectile (bullet). Blank (imitation) firing guns can appear remarkably realistic to their real counterparts. Many blank guns look like authentic firearms. However, blank guns are not designed, at time of manufacture, to fire a projectile (bullet) from the barrel. In fact, at time of manufacture, many of these barrels are sealed or plugged, the chamber may be designed shorter so not to allow a live cartridge to seat properly, and vent holes are sometimes located on the barrel to disperse the energy that results from firing a live cartridge (preventing this energy from being dispersed from the end of the barrel). Blank ammunition is designed with a case, propellant, and primer but no projectile (bullet). When discharged, the blank cartridge has the potential to produce a flash, an explosive sound, and the blank gun cycles in similar fashion to an authentic firearm. These blank guns are also sometimes referred to as imitation guns, starter guns, or even "Hollywood" prop guns. In this research project, we examined two blank firing guns and two authentic firearms using a sound meter and high-speed photography. Two blank-firing handguns, one representing a revolver (Bruni Magnum) and the other a semi-automatic pistol (EKOL), were tested for sound generation and light generation. Two sound meters (Extech Digital Sound Meter and Ametek MK-3) and a high-speed camera (Phantom V711) were employed to record the data. This data was compared to similar-looking authentic firearms firing live ammunition (Beretta 92FS pistol and Smith & Wesson 586-1 revolver). In addition, the two types of blank ammunition were disassembled for comparison to live ammunition to examine the powder components.

#### Firearm, Blank Gun, Ballistics

## B69 The Repeatability of Ridge Width Measurements for Latent Fingermark Aging Studies

*Josep De Alcaraz-Fossoul, PhD\*, University of New Haven, West Haven, CT 06516; Michelle Mancenido, PhD, Arizona State University, Glendale, AZ 85306-4908; Carme Barrot, PhD, University of Barcelona, Barcelona 08036, SPAIN; Sara C. Zapico, PhD, FL International University, IFRI, Miami, FL 33199; Katherine A. Roberts, PhD, Hertzberg-Davis Forensic Science Center, Los Angeles, CA 90032-4210; Melissa J. Sirard, Chandler, AZ 85226; Anahi Barraza, Phoenix, AZ 85033; Sara R. Anderson, Arizona State University, Glendale, AZ 85306-4908; Natalie Rivera Cardenas, BSc, Arizona State University, Glendale, AZ 85306*

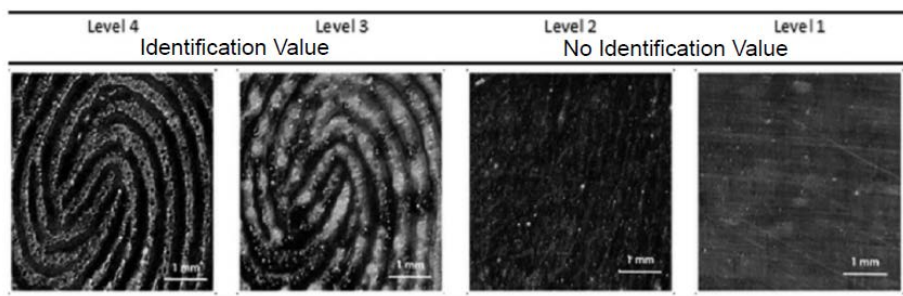
**Learning Overview:** After attending this presentation, attendees will learn about the reliability of manually-collected measurements of latent fingermarks' ridge widths. Additionally, they will be introduced to the statistical methods used in the analysis.

**Impact on the Forensic Science Community:** This presentation will impact the scientific community by demonstrating the robustness of the method used to measure topographical features with the goals to introduce automation in the future and use ridge widths as a parameter to estimate degradation patterns over time.

Fingermark identifications and analyses have been performed for over 100 years and are still a valuable forensic tool. However, the issue of when a latent fingermark was left behind is a recurring concern in the criminal justice system. Currently, there are no standards for estimating the time of deposition of a latent fingermark. To address this challenging matter, researchers have been seeking visual ways to model natural degradation patterns under the effect of different environmental conditions.<sup>1-4</sup> A reliable, portable, and inexpensive method to reveal the age of fingermarks could prove useful to crime scene investigations as it would exclude potential suspects if the estimated time of deposition is inconsistent with the commission of the crime. According to past research, changes in fingermark ridge widths over time could be used as a possible parameter to model the natural aging process of latent fingermarks.<sup>5,6</sup>

The purpose of this project was to statistically analyze the consistency of ridge width measurements across examiners obtained from different types of fingermark imprints. Measuring ridge widths manually, in the absence of an automated tool, is a highly subjective process. Therefore, determining the deviation of measurements on the same exact fingermarks becomes crucial. Repeatability, precision, and accuracy of each examiner was established.

The experiment involved testing the repeatability of measurements within and across four independent examiners. These consisted of three trained students and one expert. Intra- and inter-variability was statistically examined on fresh latent fingermarks powdered with titanium dioxide and carbon black, as well as on inked prints (flat and rolled) of the same fingers. A total of 90 fingermarks were examined using digital imaging software from 10 different donors. A statistical randomization schema for sample analysis was designed that resulted in a simplified version of data collection and a systematic approach to every measurement. Data was collected in three random rounds for each image. An examination of the quality of fingermarks [Fig. 1] was performed to evaluate how it could influence the accuracy of ridge width measurements, as shown:



[1] Categories of latent fingermark quality defined as a function of "levels of ridge detail"

For image quality, attribute agreement analysis was performed to confirm the agreement of examiners with themselves (intra-variability) and with each other (inter-variability). A method that is often used in agreement analysis where ratings are ordinal calculates Fleiss' Kappa, which tests for significance of intra- and inter-variability using asymptotic normal theory. Results suggested that examiners agreed with themselves and with each other. This suggests that intra-variability was not significant. Further, Fleiss Kappa statistics were calculated to confirm the agreement among examiners for each quality level. In this data set, examiners rated the prints at levels 3 and 4 (none were rated at 1 or 2). Results implied that the examiners agreed with each other for the quality level.

For the actual ridge width data, 30 points of measurement were distributed over 6 regions away from any identifiable minutiae [Fig. 2]. The same exact points were measured for all different types of prints. These points were cross-sections of ridges measured in pixels.



Fig. 2. Example of an inked fingerprint image depicting the 30-point measurement distributed over 6 regions

Statistical analysis [Fig. 3] revealed that there were no significant differences within and across examiners (i.e., intra- and inter- variability). Thus, there was a consensus of measurement accuracy and precision among them proving the consistency and reproducibility of the method used. However, data consistently revealed ridge width differences between the latent and inked groups, and within the inked subgroups (flat vs rolled). These differences could be explained by the nature of the fingerprints themselves (latent vs. visible) and the deposition method for the inked prints (plain static vs. rolling motion). No significant differences were detected between the white and black powdered subgroups.

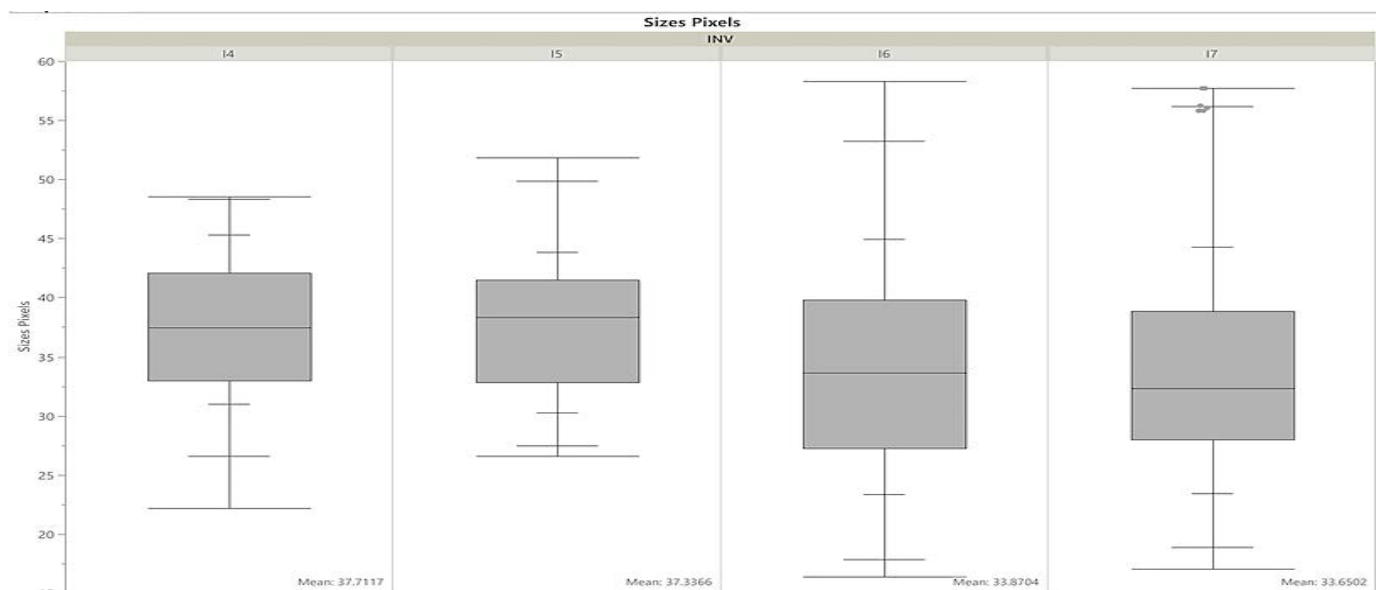


Fig. 3. Boxplot of overall raw data (ridge widths in pixels) per examiner.

This research provides evidence that changes in ridge width as result of natural degradation over time could be measured in a consistent manner. It contributes to the ongoing search for an inexpensive visual method for estimating the age of latent fingerprints and provides a basis for future research and automation for the analysis of topographical features.

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#### Ridge Width, Morphometrics, Degradation

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### **B70 A Comparison and Analysis of New Impression Casting Mediums to Dental Stone and Plaster of Paris**

*Alexander J. Colla, MFS\*, National University, Forensic Sciences Program, La Jolla, CA 92037; Ismail M. Sebetan, MD, PhD\*, National University, La Jolla, CA 92037-1011; Paul Stein, PhD\*, National University, La Jolla, CA 92037*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the qualities and positive attributes of new impression casting media compared to Dental Stone and Plaster of Paris. This study evaluated QuikCrete, Epoxy Resin, Caulking, Insta-Foam, and Silicone Rubber in comparison to the gold standard presented by Dental Stone and Plaster of Paris.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring the possibility that there are better casting materials available today. This will improve the quality of three-dimensional footprint impressions and ultimately improve the making of probative match decisions (or exclusions) based on impression evidence.

This study theorized that there currently are five new casting media that would be comparable or even superior to Dental Stone and Plaster of Paris. Each footprint impression was made in a confined path in a container with a soil substrate. A male volunteer weighing 185 pounds, made a typical walking motion in the soil to create shoeprint impressions. The first objective was to examine each material's ability to retain specific tread details from the worn shoe. Two specific details were selected from the shoe and each material was evaluated on its ability to retain those details. The second objective was to evaluate each material's preparation time, ease of use, and overall drying or casting time. The third objective was to analyze each material on nine points based on the following characteristics: a material that can reproduce very fine detail, have the viscosity to flow evenly throughout the impression and not be absorbed, cleaned without loss of detail, reasonable in cost, easily obtainable, easy to mix and use, set in a reasonable amount of time, not require special equipment or procedures, and not have a limited shelf-life (Bodziak, 2000).

The first objective concluded that only Dental Stone and Plaster of Paris were able to retain the finer details of the shoeprint impression, while the other materials retained only the simplest features. The second objective resulted in all materials having the same ease of use, a difference in preparation time by a margin of minutes, with drying time reflecting the greatest difference. The results from the third objective indicated that Dental Stone and Plaster of Paris met the established nine-point criteria. Insta-Foam finished with seven out of nine attributes. QuikCrete and Caulking both possessed six out of nine attributes. On the low end both Silicone Rubber and Epoxy Resin obtained three out of the nine attributes. The low scores for Silicone Rubber and Epoxy Resin were caused by the materials intercalation with the soil, making it impossible to separate the impression without severely ablating or destroying the impression.

Overall, this study revealed no definitive advantage of the other media compared to Dental Stone or Plaster of Paris. Although each material did have certain positive attributes that will need to be investigated further, they were still found lacking in overall performance. This study concluded that Dental Stone and Plaster of Paris remain the epitome of three-dimensional casting media used. These findings can be used to select the most appropriate casting mediums for impression evidence.

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#### **Casting Media, 3D Shoe Impressions, Dental Stone-Plaster of Paris**



### B71 The Dealkylation of Secondary Amines in the Presence of Botanical Materials

Katherine E. Eames\*, Farmington, NY 14425; Shana M. Hogg, PhD, Center for Forensic Science Research and Education, Willow Grove, PA 19090; David Buzby, BS, NMS Labs, Willow Grove, PA 19090; Francis X. Diamond, BS, Willow Grove, PA 19090; Heather L. Harris, JD, Glenside, PA 19038

**Learning Overview:** After attending this presentation, attendees will understand the risk of false positive results associated with analytes containing secondary amines in the presence of certain botanical materials when using an acid/base extraction and gas chromatography/mass spectrometry (GC/MS) analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying a conversion of secondary amine containing compounds into possible controlled substances that could generate false positive results in forensic drug chemistry testing of botanical materials.

Botanical materials, such as marijuana and damiana, present complicated matrices to a forensic drug chemistry lab. The use of n-propylamphetamine as an internal standard for analysis through an acid/base extraction followed by Gas Chromatography/Mass Spectrometry (GC/MS), exhibited the dealkylation of the amine in the presence of certain botanical materials yielding amphetamine. Studies into the reaction parameters impacting the conversion suggest the potential for other n-substituted amphetamines and structurally similar secondary amines to dealkylate under similar conditions. This conversion may result in the production of compounds regulated by either state or federal law. Therefore, their presence in such samples can result in legal penalties despite the ambiguity of the source.

Botanical materials of a variety of species and families common in drug lab cases or everyday uses including Marijuana (*Cannabis L. sativa*), Damiana (*Turnera diffusa*), Marshmallow Leaf (*Althaea officinalis*), Kratom (*Mitragyna speciosa*) and numerous herbs from the *Lamiaceae* (mint) family were tested to determine with which materials this dealkylation may occur. Reference standards of common plant compounds such as chlorophyll A and B were also prepared as 1 mg/mL solutions in methanol. In addition to n-propylamphetamine, various other substituted amphetamine compounds were also tested including, methamphetamine, n-ethylamphetamine, n-butylamphetamine, ephedrine, mephedrone, and methcathinone.

An acid/base extraction was performed on the different botanical materials in water spiked with the various secondary amine compounds and tested by Gas Chromatography/Mass Spectrometry (GC/MS). An Agilent® DB-1 Capillary Column, 12 m X 200µm X 0.33 µm was used. Injection volume of 1 µL, splitless injection mode with constant pressure at an inlet temperature of 265°C and detection temperature of 300°C was used for all analyses.

First, all botanical materials were tested in the presence of n-propylamphetamine. Conversion to amphetamine was observed with Damiana leaf and leaf powder, Marshmallow leaf, chlorophyll A and chlorophyll B. The dealkylation of methamphetamine, n-ethylamphetamine, n-butylamphetamine, ephedrine and mephedrone was tested only with Damiana and Marshmallow leaves and was observed with both botanical materials for all secondary amine compounds in this study.

Mechanistic investigations indicate the potential for an oxidative n-dealkylation pathway catalyzed by a metal centered porphyrin ring. The difference in catalysis by different botanical materials can be explained by the degradation of this porphyrin structure as the plant ages after being harvested.

The dealkylation of secondary amine containing drugs in the presence of certain botanical materials raises legal questions regarding the source of the resulting primary amine-controlled substances identified during analysis. Identifying the factors and causes of this reaction are important for quality control in forensic drug chemistry labs.

#### False Positive, Botanical Materials, GC/MS



### B72 Practical Techniques for the Enhancement of Textile Impressions in Vehicle Surface Coatings

*Jessica E. Hovingh\**, State College, PA 16803; *Abigail J. Bender, BS\**, Pennsylvania University, University Park, PA 16802; *Ralph R. Ristenbatt III, MS*, Pennsylvania State University, University Park, PA 16802

**Learning Overview:** After attending this presentation, attendees will gain an appreciation for the challenges associated with detecting and photographing three-dimensional fabric impressions in vehicular surfaces and will learn practical enhancement and recording techniques.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by equipping practitioners with techniques for enhancing and capturing fabric impressions in vehicle surfaces as demonstrated by use of common materials and studying various techniques.

Several different physical and material traces can be generated because of pedestrian-vehicle collisions. Many traces recovered from the scene are often categorized as so-called “trace evidence.” These traces frequently originate from the vehicle and can include fragments of paint, glass, metal, plastic, and other synthetic materials; patterns from the vehicle’s tires or other structural components may also be found, often on the roadway and occasionally on the victim or their clothing. If a vehicle is recovered, physical evidence transferred to the vehicle may be discovered; these traces can include biological substances (blood, saliva, hair, skin, and other tissues), pieces of clothing fabric and fibers. Occasionally, three-dimensional (3D) pattern impressions from clothing fabrics may be found on the vehicle, often in the vehicle’s surface coating (“paint”). While these patterns may be present, they are often overlooked or considered unsuitable for further analysis due to the difficulties associated with detecting, visualizing, and recording the impression, especially when the indented substrate is white or light in color. This study aims to investigate familiar and novel methods to optimally visualize and record such impressions.

Consistent 3D fabric impressions like those seen in pedestrian-vehicle collisions were generated using a large pendulum impact device. A simulated knee, consisting of a domed weld cap covered in 1/2-inch thick foam, was fixed to the end of the 6-foot pendulum arm. A square piece of washed Levi’s 550™ denim jeans was then fastened over the foam. The pendulum arm was equipped with a quick-release, permitting it to swing only under the influence of gravity. The simulated denim-covered knee was then released, allowing it to impact a section previously removed from a vehicle, resulting in the generation of a 3D textile impression. All variables were held constant (pendulum drop height, simulated kneecap, fabric, and vehicle sections) to produce consistent impressions, which afforded ideal comparison of the enhancement methods.

Several enhancements and recording methods were evaluated in this study. Many familiar tools and techniques, including optical filters, fingerprint dusting powders and small particle reagent, showed potential for enhancing 3D impressions. To identify a recommended approach for fabric impression enhancement, the success of the evaluated enhancement methods was compared. Popular techniques, such as the application of fingerprint dusting powder with a feather brush were determined to be suitable for detecting and enhancing impressions in some vehicle surfaces. Other powders, including fluorescent fingerprint dusting powders and small particle reagent, also provided enhancement. Visualization can be improved by removal of excess powder. Transfer techniques, such as lifting using gelatin lifters and Mikrosil™ casting, can be implemented. Use of specialized instrumentation may also provide a supplemental method of impression recording. This study investigated the use of the Zygo® Nexview™ 3D Optical Surface Profilometer to scan fabric impressions. The profilometer scans the vehicle surface, recording the fine detail of the impression. Impression depth can also be determined with this instrument, providing additional useful information to analysts.

#### 3D Impression Evidence, Enhancement, Collision Investigation



## B73 A Comparison of D-Amino Acid Levels in Historical Parchment and Leather

Krysalyn C. Gerhardt, BS\*, *The George Washington University, Washington, DC 20007*; Mehdi Moini, PhD, *The George Washington University, Washington, DC 20007*

**Learning Overview:** After attending this presentation, attendees will understand the differences in racemization rates of certain amino acids among historical parchments and leathers as old as 5,000 years old. While parchment shows exceptionally high levels of D-tyrosine and relatively high levels of D-phenylalanine, the levels in leather are virtually nonexistent for both. This is attributed to differences in processing and preservation methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enhancing the knowledge of amino acid racemization behavior in historical parchment and leather. Association of the proteinaceous artifacts with carbon dated human remains establishes a point of comparison and allows the amino acid levels to be investigated for external contributors to their conditions. Ultimately, the cause of unusually high levels of D-tyrosine in historical parchments will be assessed.

Recently, Moini et al. introduced a new racemization dating technique which implements capillary electrophoresis mass spectrometry (CE-MS) analysis of HCl hydrolyzed proteinaceous specimens to determine D to L ratios of various amino acids. Moreover, the technique uses proteomics to identify and quantify the specimens' impurities, if any, using only ~50 µg of protein. Previously studied were the effects of environmental factors, such as extreme pH and temperature, on racemization rates of silk amino acids. Compared to their naturally aged counterparts, the D/L ratios of aspartic acid, phenylalanine and tyrosine increased significantly.<sup>1</sup> More recently, a combined 18 samples of parchment and leather received from various institutions in the world were dated to range from modern to 5000 years old. Some leather samples were dated by association with carbon dated human remains. To confirm the age of the leather, the amino acid racemization (AAR) dating technique was used. Notably, the analysis of parchments revealed exceptionally high levels of D-tyrosine and relatively high levels of D-phenylalanine, while that of leather revealed miniscule levels of both.

Both parchment and leather are made from animal skins which are soaked in lime and scraped to remove the hair, washed, then pumiced to smooth for writing. Another method uses salt and flour rather than lime. Leather is then tanned—a process which alters the protein structure of a skin and reduces its susceptibility to bacteria and deterioration. Parchment especially is prone to degradation if not preserved almost immediately after the skin is removed from the animal. Therefore, this investigation of unusually high levels of D-tyrosine in parchment focuses on chemical and biological phenomena that are known to enhance the formation of D-tyrosine.

First, the effects that historical parchment preservation techniques have on the racemization of tyrosine and phenylalanine were studied. Research demonstrates that the presence of acetic anhydride significantly increases the racemization rate of certain amino acids, including tyrosine, in NaOH and acetic acid solutions.<sup>2</sup> It is possible that acetic anhydride is used in the production or preservation processes or may be a byproduct of the chemicals used. This theory may be tested by applying to parchment the chemicals known to increase racemization of tyrosine and those used in the preservation process. A comparison can determine if the combination of chemicals will produce the same effect as acetic anhydride. The results of this investigation will be presented.

Secondly, biofilm formation was investigated in relation to parchment. Certain bacteria or microbial communities can lead to the formation of biofilms; the bacteria of biofilms protect themselves from being chewed up by other bacteria by incorporating D-amino acids which are prone to enzymatic proteolysis. A concentration of 5nM D-tyrosine proves to be effective in inhibiting biofilm formation with respect to the bacteria *Pseudomonas aeruginosa*.<sup>3</sup> These proteolytic bacteria are the most common bacteria found in parchment and leather.<sup>4</sup> While biofilms create an infinitesimal layer compared to the parchment itself, the bacteria could run throughout the artifact. The samples will be examined for the presence of these bacteria using proteomics data. While the synthesis for most D-amino acids is due to the enzymatic effect of racemase, the same study suggests D-tyrosine in parchment to be produced primarily through the effects of the liming process and lengthy exposure to alkalinity.

Links between these studies and ours of parchment are still under investigation. With a better understanding of this scientific anomaly, the dating of these historical artifacts could be improved.

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### Amino Acid Racemization Dating, Parchment and Leather, CE/MS and Proteomics

### B74 The Application of Probabilistic Genotyping Software Analysis for Mixture Deconvolution Using a New Massively Parallel Sequencing (MPS) Panel for Microhaplotypes

Drew A. Bader, AB\*, Washington, DC 20007; Fabio Oldoni, PhD, The George Washington University, Washington, DC 20007; Charles H. Brenner, PhD, Oakland, CA 94611-1336; Kenneth Kidd, PhD, Yale University School of Medicine, New Haven, CT 06520; Daniele S. Podini, PhD, Department of Forensic Science, Washington, DC 20007

**Learning Overview:** After attending this presentation, attendees will have a better understanding of Probabilistic Genotyping (PG) software for mixture deconvolution utilizing Microhaplotype (MH) markers.

**Impact on the Forensic Science Community:** The presentation will impact the forensics community by demonstrating the efficacy of semi-continuous and continuous PG models when applied to deconvolution of complex mixtures generated using Massively Parallel Sequencing (MPS) of Microhaplotype (MH) loci.

Microhaplotypes are novel markers defined by two or more single nucleotide polymorphisms (SNPs) located within less than 300 nucleotides from one another. These SNPs can be associated in multi-allelic combinations within a locus, thereby generating a haplotype.<sup>1</sup> MHs are useful markers for human identification and bio-geographic ancestry prediction, while also enabling enhancing mixture deconvolution capabilities when coupled with probabilistic genotyping considering the abundance of information provided by these markers.<sup>1-3</sup>

A novel panel of 74 MH loci was developed and implemented on the Ion Chef/Ion S5™ (Thermo Fisher Scientific) massively parallel sequencing (MPS) platform and its sensitivity assessed using 2ng to 25 pg input DNA.<sup>4</sup> This sensitivity study not only establishes the limitations of the method but also provides an essential means of determining the stochastic behavior of the minor contributor for the continuous model of probabilistic genotyping. Deconvolution was explored using artificial mixtures of two to five contributors of different contribution ratios and ancestries, which were genotyped in parallel for MPS of MHs. The significance of the deconvolution was quantified by the PG software with likelihood ratios (LRs) which evaluate the strength of two competing hypotheses to quantify which hypothesis provides the best explanation for the data present in the mixture.

The two distinct models of probabilistic genotyping available to assist in mixture interpretation utilize different approaches to address deconvolution. The semi-continuous model represents a binary-like approach in that alleles are absent or present with the occurrence of drop-in and drop-out considered during the binary statistical evaluation.<sup>5</sup> However, this model requires manual assessment of contributor number, which in complex mixture it is essentially unknown and requires an assumption be made by the analyst. The continuous model, on the other hand, is non-binary in that it does not examine mainly the presence or absence of peaks but also uses the peak heights and stochastic variability in its deconvolution process.<sup>5</sup> Unlike the semi-continuous model, the continuous model used in this study does not require a number of contributors assumption by the analyst and instead only requires an assessment of stochastic behavior through a sensitivity study. In this study we evaluated the potential application of the semi-continuous LRMixStudio (version 2.1.4) and continuous MixtureSolutions (version 18-6-20) PG software for the analysis of mixture profiles using MH loci.

LRMixStudio produced LR values for the minor contributor(s) that were consistent with the expected LR range of MH profiling. In particular, an LR value of  $9.90 \times 10^{14}$  was obtained for a 10:1 mixture while,  $5.26 \times 10^8$  and  $1.55 \times 10^9$  for the two minor contributors at a 10:1:1 ratio. Finally, LR values of  $2.57 \times 10^4$ ,  $1.28 \times 10^5$  and  $8.53 \times 10^7$  were generated for each contributor, respectively at 10:1:1:1 mixture ratio. MixtureSolutions was able to produce LR value of  $2.0 \times 10^{24}$  for the minor contributor at 1:10 ratio while a 10:1:1 mixture produced a  $6.0 \times 10^{40}$  LR for the first minor contributor and a  $4.0 \times 10^{46}$  LR for the second minor when a stochastic ratio of 6.1 was applied to the analysis. A 5:1:1:1 mixture was able to produce the following LRs for each minor contributor: of  $4.0 \times 10^{54}$ ,  $4.0 \times 10^{63}$ , and  $2.0 \times 10^{71}$  applying a stochastic ratio of 6.6 for the statistical analysis.

Preliminary results indicate that MH loci are amenable to PG software analysis, which enhances mixture deconvolution capabilities and assists forensic DNA practitioners in the interpretation of higher-order mixed MH profiles with promising LR values for the inclusion of minor contributors within imbalanced mixtures. This analysis represents the first iteration of mixture deconvolution employing MH specifically with PG and will continue to develop as the software and methods improve but demonstrate the viability of this approach to addressing the issues associated with complex mixture interpretation.

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#### Microhaplotypes, Probabilistic Genotyping, Mixture Deconvolution



### **B75 Quantifying the Impact of Post-Validation Modifications to Forensic Statistical Tool (FST)**

*Nathaniel D. Adams, BS\*, Forensic Bioinformatic Services, Fairborn, OH 45324; Stephen Lorenz, Clarkson University, Potsdam, NY 13699; Marzieh Babaeianjelodar, MS, Clarkson University, Potsdam, NY 13699; Jeanna N. Matthews, PhD, Clarkson University, Potsdam, NY 13699; Dan Krane, PhD, Dayton, OH 45435*

**Learning Overview:** After attending this presentation, attendees will better understand the significance of seemingly minor algorithmic and source code modifications made to a probabilistic genotyping system in use for over seven years, the Forensic Statistical Tool (FST), developed by the New York City Office of the Chief Medical Examiner (OCME).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by quantifying, characterizing, and discussing the impact of post-validation software modifications made to FST on likelihood ratios reported by the system. By examining over 400 samples used for validation (with comparisons to over 1,000 known non-contributors), these relevant communities will be better able to evaluate the impact of source code modifications on likelihood ratios in reported results.

FST was brought online for casework in April 2011, and has been used over in over 1,300 cases in about fifty jurisdictions. In April 2011, FST was brought offline for software maintenance, at which time its likelihood ratio algorithm and corresponding source code were modified. Rather than including all loci in all calculations, FST's post-modification version removes data for loci where frequencies of observed alleles across all replicate amplifications summed to  $\geq 97\%$  in any of FST's four reference populations.

Several factors (including the use of minimum allele frequencies, multiple amplifications, four reference populations, and the occurrence of drop-in) resulted in more than 23% of evaluated 2- and 3- person mixtures to have one or more loci removed from consideration after the source code was changed.

For reporting FST results in casework, the OCME utilizes a verbal equivalency scale for reporting. Categories of support for the prosecution or defense hypotheses include limited, moderate, strong, and very strong. This presentation will describe changes in verbal equivalency category for more than 100 Hp-true and 100,000 Hd-true analyses affected by locus-dropping behaviors.

Changes to the FST software are followed by "performance check" regression tests. In 2011, these checks compared pre- and post-modification likelihood ratios for twelve samples, two of which exhibited locus-dropping at one locus each. In 2013, these checks were expanded to include four additional samples, none of which exhibited locus-dropping behaviors. This presentation will describe changes to the likelihood ratios for known contributors to more than 100 mixtures where the locus-dropping behavior occurred.

Findings will be described as both general trends and breakdowns of trends by sample category, (e.g., number of contributors, amplification protocol, and template amount). To-date, no public study has been described for the modified FST algorithm or any comparison of pre- and post-modification versions of FST.

#### **FST, Validation, Probabilistic Genotyping**



### B76 How to Decide How Many Contributors Are in a Mixture

Charles H. Brenner, PhD\*, Oakland, CA 94611-1336

**Learning Overview:** After attending this presentation, attendees will appreciate how a defining "contributor" is not obvious, but once the word is understood in a sensible and practical way, it is possible to decide how many contributors are in a DNA mixture.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting usefully clear thinking about difficult and critical problems in evaluating DNA evidence.

All actual mixture programs calculate in a way that utilizes a "number of contributors" parameter. If one assumes an inappropriate number, the resulting final computation of evidential strength is wrong, perhaps by a lot. Several published studies suggest various ideas about estimating the number, but none of them even claim to be nearly always right.

There is a way. If the mixture program is designed to calculate likelihood ratios via explicitly calculating both numerator and denominator (prosecution and defense) likelihoods  $L_p$  and  $L_d$ , and can calculate each of those likelihoods for a range of numbers of contributors --  $L_p(n)$  and  $L_d(n)$  for  $n=1, 2, 3, \dots$  contributors -- then examination of individual likelihoods says everything there is to know about the appropriate number of contributors. How to use the information may be debated, but a simple answer is to choose the numbers  $n$  and  $m$ , for each of prosecution and defense independently, that maximize the respective likelihoods  $L_p(n)$  and  $L_d(m)$ .

Note that this method subsumes the possibility of different numbers for prosecution and for defense. That's a possibility that the authors discussed in early papers that has since been given lip service as theory but in practice it has not only been overlooked, it is precluded by some popular advanced mixture programs.<sup>1,2</sup>

A futile body of literature and software has appeared for guessing the number of contributors in a DNA mixture. Ideas range from merely counting observed alleles per locus, to black box pattern recognition learning programs. All have a substantial error rate. None of them explore what it means to be a "contributor," hence all implicitly assume that the definition is obvious.

Obvious? Does "contributor" obviously mean someone who is the ultimate source of even a single cell? That's a bright-line definition (and the definition implied by allele-counting guesses, which assume a binary mixture model) but not a useful one. An inconsequential contribution is irrelevant to a mixture program. Methods tested against real but lab-concocted data embody a "ground truth" concept of contributor. That's appealing for developing and testing the algorithm but for real casework "ground truth" is not merely elusive, it's undefinable. One confounding concern among several is that low-level "contributors" exist or not according to the choice of detection threshold. Another interesting and subtle complication is the possibility that the mixture looks like a different number of contributors depending whether the suspect is assumed present or not. That means that, as discussed above, for prosecution and defense there are two *different* numbers, a situation that no analysis looking at the mixture data alone can possibly get right.

In summary, the essential first step in attacking the "number of contributors" problem is to define the terms in the relevant and practical sense. Since the purpose of the answer is to feed it into a computer program, by "mixture" we don't mean a collection of chemical DNA; we mean a collection of numbers, the measurements that are fed into the program. By "contributor" and the number thereof, the appropriate operational definitions are based on the operation of an adequate mixture model. That is, they are whatever a good continuous-model mixture program implements them as. Sensible definitions as a foundation guide thinking onto the right track.

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2. Peter Gill, Charles Brenner, John Buckleton, *et al.*, "DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures," *For Sci International* (2006)160(2-3), 90-101

#### Mixture, Contributor, Definition



### **B77 A Logistic Regression Approach for Combining Likelihood Ratio (LR) in the Field of DNA Mixture Interpretation**

*Eugenio Alladio, PhD\**, Department of Chemistry, University of Torino, Torino 10125, ITALY; *Monica Omedei, PhD*, Centro Regionale Antidoping "A. Bertinaria", Orbassano 10043, ITALY; *Selena Cisana, PhD*, Centro Regionale Antidoping A Bertinaria, Orbassano 10043, ITALY; *Denise Caneparo, MS*, Orbassano, ITALY; *Paolo Garofano, MD, PhD*, Laboratorio Genetica Forense - CAD, Orbassano (TO) 10043, ITALY; *Tereza Neocleous, PhD*, School of Mathematics and Statistics, Glasgow G12 8QQ, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will understand the critical role DNA typing interpretation plays in courtrooms and how the outcome can change dramatically if the evidence collected at a crime scene is considered as a single item instead of different parts of a whole, and therefore offering different degrees of reliability.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that logistic regression could act as a valuable tool for the combination of likelihood ratios (LRs) provided by various biostatistical software and approaches. It returns a unique LR value that eases the interpretation process and allows combination of the various LR results with parameters such as the degradation index and the overall amount of DNA to be amplified.

Due to different interpretation procedures, Low-Template DNA (LT-DNA) mixture profiles obtained from the crime scene represent the most challenging situation for the DNA analyst. Their interpretation may prove very difficult, especially if several variables are present. These variables may be of different nature (e.g., random sample degradation, contamination, etc.). Furthermore, another source of complexity to interpretation of DNA mixtures is the fact that there are three main models that can be used when interpreting data obtained from electropherograms (epg): the binary approach (currently obsolete), the semi-continuous approach, and the fully-continuous approach. These models present different degrees of difficulty in terms of application and interpretation. Despite the forensic community having proposed several recommendations over the past few years, a standardized, "universal," and rigorous approach to LT-DNA mixture analyses has still to be defined.

The main aim of this study is to build a generalized, comprehensive approach to combine the likelihood ratio (LR) results that are provided by the different probabilistic approaches (and corresponding different software) for DNA mixture interpretation.

Several *ad hoc* DNA 2- and 3-person mixtures (already employed for validation purposes) were analyzed at the Laboratory of Forensic Genetics of the Regional Antidoping and Toxicology Center "A. Bertinaria" (Orbassano, Italy) by means of different probabilistic software involving both the semi-continuous (or qualitative) and the fully-continuous (or quantitative) models. In particular, software such as LRmix Studio, Lab Retriever, DNA•VIEW®, EuroForMix and STRmix™ were employed in this study, but the developed approach can be extended to an unlimited number and kind of different software. Logistic regression approaches were used to combine the different LR values, together with the degradation index and DNA quantification parameters. Furthermore, a simple R Shiny app has been developed to allow analysts to deal with this kind of data.

#### **DNA Mixtures Interpretation, Logistic Regression, Likelihood Ratio**





### B78 Evaluating Likelihood Ratio Variability of Major Component DNA Profiles in Complex Mixtures

*Steven Weitz, MS\*, ATF, Beltsville, MD 20705; Todd W. Bille, MS, Bureau of ATFE, Ammendale, MD 20705; Gregory A. Peiffer, PhD, Bureau of ATF, Beltsville, MD 20705-1250; John S. Buckleton, PhD, Auckland, NEW ZEALAND; Jo-Anne Bright, PhD, ESR Ltd, Auckland, NEW ZEALAND; Michael D. Coble, PhD, University of North Texas Health Science Center, Fort Worth, TX 76107*

**Learning Overview:** The goals of this presentation are: (1) to determine the robustness of likelihood ratios of major component DNA profiles in complex mixtures with an uncertain number of contributors, and (2) to develop a quantitative method for determining when a major component DNA profile can be confidently deconvolved using probabilistic genotyping.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering one method that can be used to quantitatively determine a major component DNA profile in a complex mixture, and to bring consensus on how uncertainty can and should be evaluated.

Validation and implementation of Applied Biosystems™ Globalfiler™ PCR amplification kit at the Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF) forensic science laboratory has resulted in an increase in the observation of complex DNA profiles. Often, these DNA profiles consist of perceived major components along with an indeterminate number of minor contributors. Deconvolution of DNA profiles using probabilistic genotyping software STRmix requires the user to provide the assumed number of contributors present in the DNA profile. Accurate determination of number of contributors to a mixture is not always possible and, in these instances, the DNA profile should not be used for comparison or statistical analysis. Complex DNA profiles with robust major components may be an exception. In this study, fourteen artificially created three, four, and five-person complex mixtures including major components were amplified using the Applied Biosystems™ Globalfiler™ PCR amplification kit at concentrations of 500pg and 1.0ng, with an additional subset being amplified at 250pg. The resulting DNA profiles were deconvolved using STRmix with the correct number of contributors and with reasonable variation to the number of contributors. All deconvolutions were compared to a database consisting of known contributors and 1,000 known non-contributors to evaluate the effect of number of contributors on calculated likelihood ratios. Deconvolution resulted in consistent characterization of the major component, and minimal variation in calculated likelihood ratios at all assumed number of contributors. As expected, reduction in the number of contributors often resulted in the exclusion of known contributors to the minor component as well as known non-contributors. Mixture proportions determined during deconvolution under varied number of contributors were analyzed and indicated that a major component is suitable for comparison if the ratio of the major proportion to the sum of the minor proportions is greater than 2:1. It is the hope of the authors that this study is viewed by the forensic DNA community as one method that can be used to quantitatively determine a major component DNA profile in a complex mixture. Additionally, it is hoped that this study will spark discussion within the community and lead to consensus on how uncertainty can and should be evaluated.

#### Mixture, Major Component, Probabilistic Genotyping



## B79 Proteomic Genotyping of Human Head Hair

Zachary C. Goecker, MPS\*, Visalia, CA; Michelle Salemi, MS, University of California, Davis Proteomics Core, Davis, CA 95616; Brett Phinney, PhD, University of California, Davis Proteomics Core, Davis, CA 95616; Glendon Parker, PhD, University of California, Davis, Davis, CA 95616; Robert Rice, PhD, University of California, Davis, Davis, CA 95616

**Learning Overview:** After attending this presentation, attendees will understand about hair shaft proteomics and the impact of chemical processing in obtaining significant powers of discrimination for sample individualization. Attendees will also learn about mass spectrometry, and its use for inferring genotype.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by shifting human identification into a dual DNA-proteomics perspective. This research will also bolster the reliability of hair analysis as a means of identification, as well as establish reliable methods in forensic proteomics.

This research explores a proteomic approach to hair analysis. Forensic hair evidence, historically analyzed by microscopic morphological comparison, has been criticized as being subjective, unreliable, and not reproducible. Mitochondrial DNA analysis has been able to remedy the reliability of hair analysis, but DNA can degrade substantially under environmental insults, particularly at the distal end of the hair shaft. Unlike DNA, protein has peptide bonds that are more resistant to cleavage. Single amino acid polymorphisms in a protein sequence may be inherited genetically, as they are a result of nonsynonymous single nucleotide polymorphisms (SNPs). These genetically-variant peptides (GVPs) offer the prospect of more discriminating analysis.<sup>1</sup> Genetic information such as SNPs can be predicted via the protein sequence and confirmed with parallel DNA sequencing. Instrumental detection can further be improved with the use of isotope-labeled peptide standards in conjunction with a targeted approach of mass spectrometry, termed Parallel Reaction Monitoring (PRM). Instrumental detection can also be improved using Data Independent Acquisition (DIA). With these analytical improvements, more GVPs can be used to help identify an individual or even classify ancestral origin given that the GVPs have different genotypic frequencies among ancestral populations. The authors hypothesize that the use of a targeted strategy for mass spectrometry in conjunction with an optimized chemical processing method will improve GVP yield and detection and therefore increase the overall power of discrimination for hair digests to over 1 in 1 billion with multiple hair shafts and over 1 in 1 million with a single hair shaft (2cm).

This research focuses on increasing both peptide yield and instrumental sensitivity by optimizing hair chemical processing and characterizing targeted standards. Hair is chemically stable and therefore physically robust due to covalent linkages such as disulfide and isopeptide bonds. Data have been obtained to optimize conditions for disulfide reduction, alkylation, and peptide digestion. Temperature, time, agitation types, and concentrations of the reagents have been tested. The three metrics used to determine the best parameters for processing are yield of peptides in the soluble fraction, unique peptide number, and the number of GVPs. Results testing 2cm of hair indicate that disulfide reduction is improved with lower temperatures, higher concentrations of DTT, and agitation by stirring. Trypsinization for 6 hours solubilizes most of the hair by mass and results in detection of more peptides. The optimized hair processing procedure, with shorter times for both reduction and digestion, has yielded improvements in detectable GVPs, and results in a similar number of GVPs compared to other approaches that rely on urea denaturation and a mass spectrometry compatible detergent. However, these other hair procedures rely on large quantities of hair. When isotope-labeled GVP standards are applied to hair digests, spectral patterns are compared between the standard and endogenous peptide to serve as a confirmation of identity. Spectral features are then used for PRM. Results from hair digests analyzed using PRM and DIA with 24 standard peptides indicate an increase in the detection sensitivity of endogenous peptides and may provide enough sensitivity to identify peptides not detected in standard modes of mass spectrometry without the use of internal standards. The PRM method in conjunction with labeled internal standards may greatly benefit detection of low-abundant GVPs. Overall, the data show that yield and detection of GVPs increases with optimized chemistry and application of a PRM or DIA strategy. Further objectives of this research include developing methods for objectively classifying human hair by ancestral group and deriving likelihood ratios to compare ancestral groups using calculated powers of discrimination.

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### Proteomics, Hair, Genetically Variant Peptides



### **B80 The Evaluation of a Quantitative Proteomic Seminal Fluid Assay for Assessing Lateral Flow Test Error Rates and Predicting Downstream DNA Profiling Success**

*Heather E. McKiernan, MSFS\*, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Catherine O. Brown, MSFS, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Kevin M. Legg, PhD, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Phillip Danielson, PhD, University of Denver, Denver, CO 80210*

**Learning Overview:** After attending this presentation, attendees will understand how a quantitative proteomic workflow for seminal fluid can be utilized to both estimate the rate of authentic false positive results associated with immunochromatographic assays as well as identify appropriate samples for subsequent genetic profiling.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a potential alternative workflow for sexual assault kit sample analysis while providing further information regarding false positive rates associated with currently employed immunochromatographic assays designed to target seminal fluid markers.

Forensic practitioners have long sought efficient and reliable means for identifying those samples that are best suited for successful genetic profiling. Traditional workflows for sexual assault kit sample processing rely upon enzyme activity and antibody-based serological tests for the detection of seminal fluid and/or saliva as well as microscopy for the detection of spermatozoa. This workflow can be laborious and costly while reliance on antibody-based serological testing can be prone to error. This has contributed to the popularity of Y-Screen assays as an alternative workflow for prioritizing sexual assault kit samples. While these Y-Screen approaches achieve rapid screening of samples for the presence of a detectible male contributor, they do not provide any serological information. As a result, samples lack what can be a critical investigative/biological context. Particularly with the introduction of more sensitive DNA profiling kits, this lack of context opens the door to alternative explanations for the presence of trace levels of male DNA (e.g., secondary/indirect transfer of skin cells).

A more sensitive and accurate technology for the confirmatory identification of seminal fluid would provide important physical evidence of sexual contact to greatly bolster the weight of even partial male DNA profiles. A particularly promising approach combines high-specificity protein biomarkers with a target-ion mass spectrometry. Applying absolute quantitation of the prostate specific antigen (PSA) and semenogelin (Sg) protein targets in the biomarker panel will enable forensic practitioners to make fuller use of serological information in their decision making on downstream analyses in order to improve the successful analysis of challenging sexual assault samples.

This research evaluated the relationship between quantitative levels of target seminal fluid peptides and the ability to generate Y-STR profiles from vaginal swabs collected at various post coital intervals. Seminal fluid protein content as determined by a proteomic assay was compared to the percent of Y-STR loci detected following genetic analysis. Based on preliminary data, there appears to be almost full concordance between target peptide concentrations and the ability to produce Y-STR profiles. These results indicate it may be possible to develop a proteomic SAK screening workflow—one which simultaneously provides a reliable means both for identifying samples likely to yield an interpretable DNA profile as well as providing serological information that speaks to the biological context of the sample.

Additionally, this quantitative proteomic assay was used to estimate the rate of authentic false positive results associated with immunochromatographic assays targeting seminal fluid proteins. Self-collected vaginal swabs collected from participants **not** engaging in barrier-free vaginal intercourse with male partners were tested using various immunochromatographic assays designed to detect both Sg and PSA. Any samples producing positive results on either immunochromatographic assay were evaluated to determine whether the target protein was present at levels above the reported sensitivity limits of the lateral flow tests. Out of the fifty (50) samples analyzed to date, 7 produced false positive results for ABACard p30 while 2 produced false positive results for RSID Semen resulting in a 14% and 4% false positive rate respectively. Interestingly, in no instance did a sample produce a positive result on both immunochromatographic assays. All cellular components of the 9 samples producing positive immunochromatographic results were confirmed sperm free utilizing Sperm Hy-Liter and fluorescent microscopy. As the proteomic assay has already been demonstrated to be more sensitive than lateral flow tests, negative results indicate that the positive immunochromatographic results were due to true false positive, non-specific binding events rather than the presence of these proteins at low levels. These data support the use of immunochromatographic assay results as presumptive rather than confirmatory.

#### **Forensic Science, Proteomics, Sexual Assault**



### B81 The Developmental Validation of a MicroRNA (miRNA) Panel for Forensic Body Fluid Identification

*Annabelle C. Campbell, BSc\*, Richmond, VA 23220; Jennifer Szekely, BS, Richmond, VA 23223; Carolyn Lewis, BS, Virginia Commonwealth University, Richmond, VA 23284; Sarah J. Seashols Williams, PhD, Virginia Commonwealth University, Richmond, VA 23284-3079; Raquel Green, BS, Richmond, VA 23220; Tracey Dawson Cruz, PhD, Virginia Commonwealth University, Richmond, VA 23284*

**Learning Overview:** After attending this presentation, attendees will understand how microRNAs (miRNAs) can be of significant value for body fluid identification in forensic casework. Attendees will be apprised of the markers that can distinguish different body fluids and understand why miRNAs may be a better molecular-based method for the identification of body fluids rather than the use of current serological tests, which are based on enzymatic activity and are often prone to false positives.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how forensic research on miRNAs continues to build evidence for their utility as forensic molecular markers.

MiRNAs are small non-coding RNAs 18-25 nucleotides in length that have been identified and evaluated as potential markers for the identification of forensically relevant body fluids. There is significant interest in the use of miRNAs for forensic casework because of their short length and high resistance to degradation. They have also been shown to co-extract and be detectable in DNA extracts, which could make the use of miRNAs a more streamlined and easily implementable molecular body fluid identification method than other described methods.

Candidate miRNAs were identified through high-throughput sequencing of the miRnome and quantitative Polymerase Chain Reaction (qPCR) panel analyses of differential expression patterns in venous and menstrual blood, vaginal secretions, saliva, feces, urine, perspiration, and semen. Identified candidate miRNAs were further validated using population sample sets from each biological fluid, ultimately identifying nine miRNAs to identify seven biological fluids. This panel of nine miRNAs includes a pair of endogenous reference markers that provide normalization of miRNA expression without evaluation of the RNA or known input quantity. This panel uses expression detected using reverse-transcription quantitative PCR (RT-qPCR) to identify and differentiate feces, urine, peripheral blood, menstrual secretions, vaginal secretions, semen, and saliva. Identification of the biological fluids was found to be reliable across population samples of mixed ages, ethnicities, and gender (where appropriate).

Panel performance for body fluid identification of miRNAs in DNA extractions was assessed and compared to paired RNA extracts and found to provide body fluid identification in 7 of the 9 body fluid samples tested. Detection in compromised samples, limit of detection, and species specificity was evaluated according to developmental validation guidelines and miRNA body fluid ID was successful despite compromising conditions and low input. However, most of the miRNAs in the panel are not human-specific.

In conclusion, the 9-miRNA panel has been shown to provide robust, accurate identification of 7 biological fluids, and continues to show potential for implementation into forensic casework.

#### Body Fluid Identification, microRNA, qPCR



### B82 Antibody-Mediated Separation of Seminal Male/Female Mixtures From Sexual Assault Samples

*Chelsie N. Testerman, MS\*, Houston, TX 77063; Jordan Cox, MS, Richmond, VA 23220; Kemper Gibson, MS, Raleigh, NC 27603; James P. Landers, PhD, University of Virginia, Charlottesville, VA 22904; Tracey Dawson Cruz, PhD, Virginia Commonwealth University, Richmond, VA 23284*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of an alternative method to traditional differential extractions using an antibody-bound, bead-mediated binding mechanism that can be performed in a microcentrifuge tube, or on an integrated sexual assault microdevice.

**Impact on the Forensic Science Community:** This presentation will impact on the forensic science community by describing a faster and more efficient way to process sexual assault samples that will also reduce the amount of time spent on mixture interpretation.

While efforts have been made to reduce the pervasive backlog of sexual assault kits, the actual laboratory process is still very time-consuming as it often involves a differential lysis step prior to DNA purification, as well as complex mixture interpretation at the end of the forensic DNA workflow. This research explores the use of an antibody-bound, bead-based capture mechanism as an alternative means of cell separation by targeting a relevant cell type (sperm, vaginal, or prostate cells). By using this mechanism for a fractional DNA extraction, cell types could potentially be separated more efficiently than the traditional differential lysis, and various specific cells can be targeted by changing the capture antibody used. In the current study, candidate sperm cell antibodies were first tested via flow cytometry to determine their binding affinity for the cell of interest. Moving forward, antibodies with the highest binding affinity for the target cell type were tested using a microcentrifuge tube-based, antibody-bound, bead capture mechanism. Downstream of separation, samples were analyzed using a traditional forensic DNA workflow, including DNA isolation, human-specific DNA quantification, multiplex STR amplification, and CE-based separation of resulting amplicons. Although sperm-specific PH20 antibody exhibited a binding affinity of 74.2% for sperm cells when tested via flow cytometry, it only captured 23.5% of the total DNA in semen samples using the bead-mediated method. Additionally, sperm-specific antibody AKAP3 bound only 0.167% of gated sperm cells, while 41.1% of the total DNA was retrieved in the bound fraction using the bead-mediated method. However, when these antibodies were tested on semen-vaginal fluid mixture samples using the antibody-bead mediated assay, STR results from the bound fractions showed that the male contributor was present in ratios that were, on average, 10-fold higher than the female. The  $\geq 10:1$  and 9.6:1 male to female ratios in the bound fractions for PH20 and AKAP3, respectively, provided for unambiguous single-source male STR profiles, rendering mixture interpretation unnecessary for these samples. Alternatively, cytokeratin-4 (CK4) antibody was used to target vaginal epithelial cells, binding 76.1% of the total DNA in vaginal epithelial cells using the antibody-bead mediated binding method. In the unbound fraction of semen-vaginal fluid mixtures, the male contributed at least 9-fold more DNA than the female to the resulting STR profiles, again providing single-source male STR profiles. This data provides evidence that the CK4 antibody may be a valid antibody for separating the female fraction of a sexual assault sample away from the male, regardless of the male cell type(s) present. Overall, PH20, AKAP3, and CK4 were able to enrich for and isolate clean single-source male profiles from sexual assault mixtures containing semen and vaginal epithelial cells. Future work on this project will include the exploration of additional antibodies for both sperm and non-sperm containing sexual assault evidence, as well as integration onto a microchip-based format. This approach could provide a faster and easier way to separate contributors that could dramatically reduce the amount of back-end mixture interpretation needed for some sexual assault samples.

#### Differential Extraction, Sexual Assault, Backlog



### B83 The Optimization and Validation of a Novel Direct-Lysis Differential Extraction Procedure

Andrew D. Ziegler, BS\*, Boston University School of Medicine, Boston, MA 02118; Anooja Rai, Boston University School of Medicine, Boston, MA 02118; Mike Yakoo, MS, New York, NY 10128; Robin W. Cotton, PhD, Boston University School of Medicine, Boston, MA 02118

**Learning Overview:** The goal of this presentation is to describe an innovative method for DNA extraction from sexual assault samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a faster method for processing backlogged sexual assault cases.

Forensic analysis of DNA from sexual assault kits is a laborious process. Samples may be a mixture of sperm and male or female epithelial cells (E-cells). Since its introduction by Peter Gill in 1985, differential extraction has remained the essential pre-PCR extraction procedure adopted by most forensic laboratories.

The differential extraction procedure relies on the differences in the protein packaging of DNA in these two types of cells. The E-cells are first lysed by the addition of SDS and Proteinase K. These reagents alone are not able to lyse the sperm cells. The mixture is centrifuged leaving E-cell DNA in the supernatant and sperm cells in the pellet. After several wash steps to remove residual E-cell DNA, the sperm fraction is then subjected to lysis using SDS, proteinase K, and dithiothreitol (DTT). DTT reduces the disulfide bonds present in the sperm nucleus, thereby releasing sperm cell DNA.

The Gill method of differential extraction, while proven to be highly effective in providing two separate fractions for a simplified interpretation of DNA profiles, requires an average of approximately six hours of an analyst's concentration. To mitigate carryover from the female fraction, the sperm cell fraction is usually subjected to multiple wash steps. Furthermore, the resulting fractions must be subjected to additional pre-PCR DNA purification procedures to remove PCR inhibitors such as SDS and Proteinase K. These steps inevitably result in some DNA loss, particularly when few sperm were initially present in the sample.

Progress has been made in developing methods that allow for PCR-ready cell lysates that do not require additional purification steps (referred to as direct-lysis methods). However, thus far, none have been proven to be viable options for use in sexual assault samples. The authors' laboratory has developed a novel differential extraction procedure that is time-efficient, less laborious, and utilizes a direct-lysis procedure requiring no further pre-PCR purification for most samples.

The procedure uses two commercially available enzymatic products (ZyGEM and AcroSolv, from ZyGEM NZ Ltd) along with a nuclease (Benzonase®) to effectively lyse cells and produce PCR-ready E-cell and sperm cell fractions suitable for downstream nucleic acid amplification. The procedure uses the different optimal activity temperatures of the enzymes to perform most of the process in a DNA extraction lab thermal cycler, requiring only a single centrifugation for the usual separation of the epithelial cell fraction from the intact sperm. No subsequent washing steps for the sperm cell fraction are required.

In typical mixtures the novel direct-lysis differential extraction procedure recovers close to 100% of the sperm DNA from freshly prepared mock sexual assault samples of varied mixture ratios. Using samples with E-cell DNA: Sperm DNA ratios of 1:1, 5:1, 20:1, and 50:1 placed on cotton swatches aged for 4 weeks, 77%-100% of sperm DNA was recovered. DNA profiles generated from the sperm fractions were almost entirely from the male contributor, as indicated by 85%-100% mean peak height contribution.

Minimal, if any, female contribution with increased epithelial cell concentration was observed. The mean percentage of female peak height carryover was 8.4% for freshly-prepared samples and 14.6% for cotton swatches at a ratio of 50:1 E-cell DNA to sperm DNA. The procedure appears to be robust in producing DNA profiles from small amounts of sperm. Data from samples where the enzymatic procedures was either scaled up or scaled down will be presented.

The novel procedure can be completed in less than two hours using an extraction lab thermal cycler and requires no additional wash steps or pre-PCR purification procedures. Additionally, the procedure reduces the number of steps and sample manipulations, decreases carry-over between fractions, and increases sperm DNA recovery. It has the potential to be a rapid, robust procedure that can be easily implemented in any forensic laboratory. The presentation will describe the procedure and show additional data from progress in the procedure validation.

#### Differential Extraction, Sexual Assault, Sperm DNA Recovery



### **B84 The Integration of a High-Resolution Melt (HRM) Curve Short Tandem Repeat (STR) Assay Into a Commercial Quantification Kit for Preliminary Mixture Detection: Getting More Information Earlier in the DNA Workflow**

*Hannah Wines, BS, Houston Forensic Science Center, Houston, TX 77030; Laura C. Oliver, MS\*, Bureau of Alcohol, Tobacco, Firearms, & Explosives, Beltsville, MD 20705; Jordan Cox, MS, Richmond, VA 23220; Darianne Cloudy, BS, Hampton, VA 23663; Sarah J. Seashols Williams, PhD, Virginia Commonwealth University, Richmond, VA 23284-3079; Steven Weitz, MS, Bureau of Alcohol, Tobacco, Firearms, & Explosives, Beltsville, MD 20705; Todd W. Bille, MS, Bureau of Alcohol, Tobacco, Firearms, & Explosives, Ammendale, MD 20705; Edward Boone, PhD, Richmond, VA 23284; Tracey Dawson Cruz, PhD, Virginia Commonwealth University, Richmond, VA 23284*

**Learning Overview:** After attending this presentation, attendees will be aware of a newly-developed modified quantification assay with high resolution melt curve analysis, coupled with back-end statistical analysis, that can be used to distinguish between single-source and mixed DNA samples early in the forensic DNA workflow.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a way to detect mixtures, and potentially provide early exclusionary data, prior to multiplex Short Tandem Repeat (STR) amplification and Capillary Electrophoresis (CE) analysis.

Currently, the forensic DNA laboratory workflow does not allow the possibility of quickly identifying exclusionary contributors or determining whether a sample is a mixture until end-point DNA profile interpretation and identification. This presents a problem for low template DNA samples, particularly those from touch DNA samples where numerous small areas of a touched item are individually swabbed to avoid inadvertently creating mixtures. A screening assay at an earlier step could be a beneficial tool allowing an analyst to determine if consumption of the sample is warranted or if swabs of different areas of the evidence item should be combined prior to STR amplification. The quantification step is the most logical place to add this screening assay, due to the multifaceted capabilities of quantitative polymerase chain reaction (qPCR) instruments and human DNA quantification kits. To this end, a high-resolution melt (HRM) curve assay was designed that could be performed on common qPCR platforms utilizing STR loci D5S818 and D18S51 as targets. The authors previously reported that analysis of HRM data from single-source samples using linear discriminant analysis (LDA) provided higher classification rates than a principle component analysis (PCA)-based method for STR genotyping and geno-grouping using the Rotor-Gene Q (RGQ) platform. Further, that work showed that the higher resolution of the RGQ improved genotyping and geno-grouping prediction accuracy rates using this method (58.9% and 81.0% for D5, respectively) when compared to the more common ABI 7500 qPCR platform (23.9% and 65.4% for D5). Thus, the current study focused on integration of the STR HRM assay into the Quantiplex<sup>®</sup> quantification kit utilizing the RGQ platform and subsequent testing of the integrated assay using mixture samples. Prior to any sample testing, the amplification reaction conditions and amplification/melt parameters for the integrated assay were tested and optimized. The final protocol for the integrated quantification/HRM assay included a reduced-volume Quantiplex<sup>®</sup> reaction, modified Quantiplex<sup>®</sup> amplification parameters, and the addition of a transition cycle and melt step (60-95°C) following amplification.

Initial testing confirmed that when the HRM dye is added to the Quantiplex<sup>®</sup> amplification reaction using the transition and melt parameters no interfering melt products are observed and that, when STR primers are added, the melt curves produced are within the expected temperature ranges and exhibit minimal background noise. Additionally, no significant differences in primary peak temperatures were noted when the samples amplified using the integrated Quantiplex<sup>®</sup>/HRM assay were compared to the optimized singleplex STR amplification/melt reaction. Interestingly, the variation of the primary melt peak temperature observed was lower using the integrated Quantiplex<sup>®</sup>/HRM assay than what was previously noted from the inter-run variation observed when singleplex amplification and melt was performed. Further, when Quantiplex<sup>®</sup> standard samples were amplified with the added STR primers and HRM dye, the human DNA quantification standard curves produced were within the acceptable quality ranges and no significant changes were noted when sample quantification values were compared to the expected values (17.74% variation from expected vs. 20.72% inter-run variation for standard Quantiplex<sup>®</sup> reactions). Thus, the integrated quantification/HRM assay was tested using 56 single-source samples and ten 2-person 1:1 mixtures. The integrated assay was able to accurately identify 100% of the mixture samples tested and 92.9% of single-source samples tested. Taking all tested samples into account, the integrated STR melt-curve quantitation assay was able to properly distinguish between single-source and mixture samples in 62 of 66 samples tested for an overall accuracy rate of 96.43%. Overall, this work provides a viable qPCR-based integrated HRM + quantification assay that can provide an analyst with indication of a mixed forensic sample early in the forensic DNA workflow.

#### **DNA Mixtures, qPCR, HRM**



### **B85 Forensic Body Fluid Identification Using Microbiome Signature Attribution Through 16S Ribosomal DNA (rDNA) High-Throughput Sequencing (HTS)**

Denise Wohlfahrt, BS\*, Virginia Commonwealth University, Richmond, VA 23284; Raquel Green, BS, Richmond, VA 23220; Angela L. Brand, BS, Southport, NC 28461; Eric A. Abshier, MS, Worldwide Counter Threat Solutions, Fredericksburg, VA 22401; Elena Martinez Planes, Virginia Commonwealth University, Richmond, VA ; Antonio Tan-Torres, MS, Virginia Commonwealth University, Richmond, VA 23284; J. Brooks, PhD, Virginia Commonwealth University, Richmond, VA ; Sarah J. Seashols Williams, PhD, Virginia Commonwealth University, Richmond, VA 23284-3079; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA 23284

**Learning Overview:** After attending this presentation, attendees will understand how microbial communities found in the body may be used to identify various forensically relevant body fluids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping forensic scientists overcome the limitations often encountered when using current serological and molecular methods. While using the microbiome as identifying markers is not new, the emergence of high-throughput sequencing allows for a more rapid and detailed analysis of these communities.

Previous studies such as the Human Microbiome Project suggest that body fluids have a distinct, stable, and distinguishable microbial signature. Identifying these microbial markers within forensically relevant fluids not only has the advantage that previously indistinguishable samples, such as venous and menstrual blood, may be separated, but these markers can also be easily implemented into comprehensive high-throughput sequencing (HTS) panels used in the forensic workflow alongside the typical human phenotypic marker characterization.

Using IRB approved collection methods, 200 urine, 200 blood, 152 feces, 149 saliva, 100 vaginal fluid, 64 menstrual secretion, and 39 semen samples were collected. Briefly, urine and semen were collected into sterile containers and stored at -80°C. Semen was dried onto sterile cotton swabs prior to DNA extraction. Venous blood was collected onto cotton swabs using a finger prick and dried at room temperature. Saliva, vaginal secretions, menstrual secretions, and feces were collected onto cotton swabs and dried at room temperature. DNA Investigator kit with the standard Forensic Casework Sample protocol on the QIAcube was used to extract blood, semen, saliva, vaginal secretions, and menstrual secretions according to manufacturer protocol. QIAamp Power Fecal DNA kit was used to extract DNA from fecal samples, and DNA Micro kit was used to extract urine samples, according to manufacturer protocol. Following extraction, V4 region of the 16S rDNA was sequenced on Miseq FGx sequencing platform following the dual-indexing protocol as described by Kozich et al (2013).<sup>1</sup> Sequences were then analyzed using mothur version 1.39.4, and statistical analysis was performed using R version 3.4.0.<sup>2,3</sup> Random subspace cross validation was performed to test the robustness of the classification accuracy.

Results of this study indicate that the microbial signatures are diverse, unique, and stable within a body fluid, common across most of the population assessed, and distinguishable from other body fluids, except for vaginal and menstrual secretions. These secretions may be distinguished from the other body sites when classified collectively as female intimate samples. Using indicator taxa, feces had a higher relative abundance of *Bacteroides* and *Faecalibacterium*, saliva had a higher relative abundance of *Streptococcus* and *Veillonella*, female intimate samples had a higher relative abundance of *Lactobacillus*, and semen had a higher relative abundance of *Corynebacterium*, when compared to each other. Random subspace classification successfully classified feces at 100%, saliva at 100%, semen at 83.3%, and female intimate samples at 94.6% accuracy, with an overall classification accuracy of 97.6%.

In conclusion, the newly developed microbiome signature-based method can identify all biological fluids except vaginal and menstrual secretions, with strong statistical certainty.

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2. Schloss, P. D. et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537-7541, doi:10.1128/AEM.01541-09AEM.01541-09 [pii] (2009).
3. R: A language and environment for statistical computing. (R Foundation for Statistical Computing, <http://www.R-project.org>, Vienna, Austria., 2011).

#### **Body Fluid ID, 16S rDNA, Microbiome**





### B86 Typing Highly Degraded DNA Using Circularized Molecules and Target Enrichment

Rachel E. Wiley, MFS\*, University of North Texas Health Science Center, Fort Worth, TX 76107; Magdalena Bus, PhD, University of North Texas Center for Human Identification, Fort Worth, TX 76107; Jonathan King, MS, Fort Worth, TX 76107; Bruce Budowle, PhD, University of North Texas Health Science Center, Fort Worth, TX 76107

**Learning Overview:** After attending this presentation, attendees will understand the limitations often encountered with current forensic genetic typing in the processing of challenged biological samples and the potential for a novel circular molecule directed method to overcome the molecular restrictions of highly degraded and damaged DNA.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing potential methods that could make human identification testing possible for samples that were previously untypable. Attendees will benefit through the presentation of alternative methods to employ that (if successful) will enhance the capabilities for forensic scientists to analyze the most severely compromised DNA samples.

Compromised biological samples often present considerable challenges for forensic genetic typing due to containing highly fragmented DNA. Current forensic typing methods do not possess the capacity to overcome the molecular limitations of low quantity and poor-quality DNA. Forensic DNA profiling employs short tandem repeat (STR) typing. However, once DNA degrades to a certain point, most STR markers fail to amplify by the Polymerase Chain Reaction (PCR). There is a considerable need to develop systems that can analyze severely challenged biological samples. First, the typing of single nucleotide polymorphisms (SNPs) can be targeted as a marker of interest as they possess an increased capacity for the typing of shorter DNA fragments due to their small amplicon size, a target size substantially smaller than that required for STR typing. Second, complete genomic amplification can be achieved within a sample through whole genome amplification (WGA). Expanding on these molecular approaches, there are two circular molecule directed techniques that are considered as a potential means to improve upon current methods for the analysis of severely compromised biological samples. Rolling circle amplification (RCA), a WGA technique, generates linear tandem copies of the circular template sequence by employing random oligonucleotide primers that bind to any template region. Amplification of a circular template essentially creates an infinitely long template. However, nuclear DNA is not found in a circular conformation and thus RCA cannot be exploited to its full potential without modification of the DNA template into a circular molecule. CircLigase™ II (Lucigen®; Middleton, WI) is an enzyme that circularizes single-stranded DNA through intrastrand ligation by the formation of a phosphodiester bond between the 5'-phosphate and the 3'-hydroxyl group. Additionally, molecular inversion probes (MIPs) are an alternative circular molecule-based enrichment approach. A MIP is a single-stranded oligonucleotide that contains two terminalized complementary regions flanking a SNP of interest (or any target sequence) and a set of internal universal PCR primer binding sites for controlled downstream amplification. The two complementary regions hybridize to the template DNA, the gap is filled via a polymerase resulting in the complementary state of the SNP of interest. The MIP will then dissociate from the target and the target site is amplified by PCR via to the internally incorporated primer binding sites. Massively parallel sequencing (MPS) can sequence the enriched circular molecules via a shotgun approach; thus, a specific target sequence is not required to obtain sequencing results.

Three candidate human identity SNPs were targeted for both circular molecule approaches. Oligonucleotide sequences of various lengths (50-mer, 100-mer, and 200-mer) were designed and synthetically generated with the candidate SNPs of interest contained within the sequence. Circularization of short synthetic DNA fragments, down to approximately 50 nucleotides (nts) in length, was achieved and amplified via RCA using the REPLI-g Mini Kit (Qiagen; Valencia, CA). Sequence data depict tandem copies of the target sequence supporting successful circularization and subsequent amplification by RCA of the circular template by the CircLigase approach. Preliminary design for the MIP approach is underway.

#### Forensic DNA, Target Enrichment, SNPs



## B87 The Bomb Maker Project: How Human Factors Affect the Characteristics and Interpretation of Explosives Evidence

*John V. Goodpaster, PhD\*, FIS Program, Indianapolis, IN 46202*

**Learning Overview:** After attending this presentation, attendees will understand: (1) the design, materials, and construction of improvised explosive devices (IEDs), and (2) how those factors may affect the way IEDs are examined in a forensic science laboratory.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by discussing the design and construction of improvised explosive devices (IEDs) and how that may influence the eventual results of laboratory examinations.

The examination of IEDs that have been either rendered safe or functioned as designed is conducted in numerous forensic science laboratories across the United States. Although IEDs vary in complexity and lethality, the typical device encountered in domestic bombings consists of some sort of rigid container, a fusing system, low explosive filler and occasionally shrapnel. The identification and comparison the explosive and components of an IED involve well-known analytical techniques that are applied to items such as tape, fuses, wires, batteries, etc. Overall, associating the materials from a device to the same type of materials possessed by a suspect has clear probative value and this is one of the main drivers for using forensic chemists in post-blast investigations.

The Bomb Maker Project seeks to study the circumstances of actual bombings and evaluate the design, materials and construction of IEDs and how those factors may affect the way IEDs are typically examined in a forensic science laboratory. Note that this study is not concerned with offender background, psychological profiles, motives or any other behavioral aspects. Furthermore, the focus of the project is on criminal bombings rather than terrorist events. Criminal bombers are much more likely to be the subject of investigations worked by state, local and federal forensic chemists in the United States.

The authors surveyed open source accounts of bombings and gathered as much information as possible regarding the explosive used, container, initiation systems, and materials used in other componentry. They have carried out internet searches of open-source news outlets, examined legal records, and searched a subscription-based service that compiles media reports of explosive incidents ([globalincidentmap.com](http://globalincidentmap.com)). Several thousand explosive incidents have been found thus far, but careful filtering is needed to remove incidents with hoax devices, bomb threats, and other events that lack an actual explosive device. Once a potential subject is located, they attempt to gather as much background information on their case as possible, including copies of the probable cause affidavit, trial transcript and any copies of appellate decisions. Ultimately, the information on attempted and/or actual bombings are described by a series of categories, as shown below:

Characteristic	Main Categories	Sub-Categories
Target	Residential	N/A
	Commercial	
	Governmental	
	Educational	
	Transportation	
	Mailbox	
	Vehicle	
Explosive Charge	Low explosive	Inorganic
		Organic
	High explosive	Inorganic
		Organic
Container	Concealment Container	Soft
		Rigid
	Confinement Container	Soft
		Rigid
Fuzing System	Non-electrical	Mechanical



		Chemical
	Electrical	Power source
		Conductor
		Load
		Switch

Given that the data consists of categorical rather than continuous variables, Categorical Principal Components Analysis (CATPCA) is being used for data analysis. This technique allows for the inclusion of categorical variables into PCA and does not assume that linear relationships exist among the data. The data is not required to be multi-variate normal in distribution. A closely related method that is also being explored is Multiple Correspondence Analysis (MCA). MCA is the equivalent of CATPCA when all the variables undergo a procedure known as multiple nominal quantitation.

The outputs of the statistical analysis include the following:

- Symmetric plots showing a projection of the observations (explosive incident) and/or the original variables in terms of the principal coordinates. This will visually indicate the degree of similarity/dissimilarity between the observations. It also indicates the extent to which the variables are correlated with one another.
- Asymmetric plots that use the principal coordinates for the variables and the standard coordinates for the observations. This will indicate how the observations are positioned relative to the category vectors.
- A quantitative report of the contributions (weight) of each variable to the principal coordinates.

The findings are then used to discern which specific explosives and components are more likely to be used in IEDs, to what extent different components are correlated with one another. Overall, this research seeks to improve the ability of practicing forensic chemists to place their findings in context more reliably, guide the selection of their methods and inform decisions regarding exemplar collections.

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### Explosives, Improvised Explosive Device, Device Components



### B88 Exploring a New Approach to Canine Training Aids for Explosives

*William A. MacCrehan, PhD\**, National Institute of Standards and Technology, Gaithersburg, MD 20899; *Craig Angle, PhD*, Auburn University, Auburn, AL; *Michele Schantz, PhD*, National Institute of Standards and Technology, Gaithersburg, MD 20899; *Paul Waggoner, PhD*, Canine Performance Sciences, Auburn, AL; *Jason Barrow, PhD*, FBI, Quantico, VA; *Kelly Van Arsdale, MS*, FBI, Quantico, VA

**Learning Overview:** After attending this presentation, attendees will understand a new method for the successful preparation of canine training aids for explosives using an odor capture-and-release technology based on Polydimethylsiloxane (PDMS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a new approach to providing nonhazardous canine training materials for hazardous substances that is being developed. This technology potentially could expand the availability of canines trained for the detection illicit substances to establish probable cause and avert terrorist threats.

A contentious issue in the training of canines to detect explosive threats is the ready availability of suitable training aid materials, particularly for TATP (triacetone triperoxide). As an easily prepared primary explosive, TATP has been used in several terrorist explosive events. Handling this sensitive, chemically unstable material requires specialized storage, transport, and expertise. Even obtaining and storing less hazardous explosives such as C-4 and TNT is a nuisance for maintenance canine training. The availability of alternative training aids (ATAs) would simplify access to reliable explosives materials.

The National Institute of Standards and Technology (NIST) has developed a promising approach to preparing ATAs based on the use of the flexible polymer, polydimethylsiloxane (PDMS). PDMS captures the odor profile of the target explosive by its rapid transport properties and affinity for organic molecules. Once “charged” with the target, the polymer slowly releases the characteristic odor of the target material for canine training. Special vapor-time measurements were used to study these capture-and-release processes for two odorants associated with the canine detection of C-4, cyclohexanone and 2-ethylhexanol. The vapor capture process takes several days at room temperature to fully charge the PDMS for these two odorants. In simulated use as ATAs, target-loaded PDMS provided a relatively steady release of odor when left out in the open for a ~4-hour period. Covering the ATA and storing it at room temperature allowed the continued re-use of the aid for additional ~4-hour periods over several months, albeit with a decline in the amount of odor released.

ATAs for TNT (TNT@PDMS) and C-4 (C-4@PDMS) were also developed and tested in canine trials. Because the volatility of TNT is so low, a more volatile impurity, 2,4-DNT is considered the primary odorant used by the canines for detection. For this experiment, elevated temperatures were studied to enhance the odor capture process. Six dogs trained on real TNT alerted to ATAs prepared with 2,4-DNT gave 100% alerts as did those charged with C-4. However, no experiments were done using the ATAs to train the canines.

In the latest collaborative experiments with Auburn University and the FBI Explosives Unit, PDMS ATAs were prepared by charging with purified TATP for one week. Thirteen experienced detection Labrador Retrievers were imprinted on TATP@PDMS. In both fixed-position scent wheel and operational searches, the canines detected real TATP at rates well over 90% of the time. Distractor experiments included 3 of the precursor chemicals used to prepare the TATP as well as 32 common materials. The sensitivity (to detect TATP) as well as the selectivity (to ignore distractor materials) was found to be excellent using this new approach to canine training aids for TATP.

#### Canine Training Aids, PDMS, TATP



## B89 A Quantitative Comparison and Differentiation of Smokeless Powders

Casey M. Brown, MPS, MVA Scientific Consultants, Duluth, GA 30096; Ryan Schonert, MPS, VUV Analytics, Cedar Park, TX 78613; Wayne Moorehead, MS, forensicTRACE, Rancho Santa Margarita, CA 92688; Jack Hietpas, PhD\*, Pennsylvania State University, University Park, PA 16802

**Learning Overview:** After attending this presentation, attendees will better understand the strengths and limitations of automated image analysis and GC/MS profiling for sample differentiation, comparison, and potential brand identification of commercially available smokeless powders.

**Impact on the Forensic Science Community:** This research will impact the forensic science community by demonstrating value of utilizing multiple techniques for the characterization and comparison of smokeless powders that can potentially be recovered from inefficient and/or undetonated improvised explosive devices.

Small arms propellants (SAP) are readily accessible and cost-effective materials that firearms enthusiasts can acquire for the legitimate assembly of ammunition. Unfortunately, the ease of access to and low cost of these materials is advantageous for their utilization in the construction of improvised explosive devices (IEDs). Typically, the SAP charge is loaded into a metal pipe (commonly steel) and sealed with screw-fit end caps. Two recent high profile domestic terrorist attacks using IEDs (Boston Marathon Bombing and NY/NJ attempted bombings) demonstrate their continued usage. Thus, there is a need to develop robust metrics for the characterization of propellants that are used as explosives as well as for comparisons between exemplar and recovered explosive residues.

The goals of the presented research are to investigate the utility of high-throughput, low cost quantitative automated image analysis and GC/MS additive of SAP for sample discrimination and potential brand identification. Ninety 1-pound canisters of SAP were purchased from local firearms stores. The samples are a wide selection of different distributors (n=8) and product brands (n=90). In addition, the sample set also has a diverse distribution of general particle morphologies (e.g., ball, disk, tube, flattened ball, etc). The first phase of the project was focused on method development for non-destructive automated image analysis. For this phase, individual smokeless powder particles were arranged on one-inch squares of clear mounting tape so that no particles were touching or overlapping. The sample preparations were then placed on a lightbox and photographed using a consumer-grade DSLR camera. Approximately 120-1600 grains/ sample were imaged. Linear calibration was performed using a NIST-certified image analysis micrometer. In addition, NIST-certified black polymer spheres of the target diameters: 100  $\mu\text{m}$ , 500  $\mu\text{m}$ , 1.5 mm and 3.00 mm were used as secondary standards to evaluate the linear calibration. Images were processed using FIJI, an open-source image analysis package. The following parameters were measured for each particle (n  $\approx$  34,000 particles *in toto*): area, perimeter, major/ minor ellipse axis, roundness, circularity, and solidity.

The large matrix of morphometric data was analyzed using the open-source statistical package R. To classify the 90 brands, 80% of the data was randomly chosen and used as a “training” set for linear discriminant analysis (LDA). The remaining 20% of the data set was treated as unknowns and “matched” to the source (brand) with the smallest Mahalanobis distance.

The second phase of this research was to assess the utility of GC/MS additive profiling. Aliquots of 20 mg for each sample were extracted with 2 mL of 3:1 methanol: n-butanol, based on the method described by Reardon and MacCrehan.<sup>1</sup> This method was chosen because it preferentially extracts the additives, leaving behind the bulk nitrocellulose. Samples were run using an HP5890 series II gas chromatograph equipped with an HP 5972 MSD and an RTX-1 column (30m x 0.25mm ID x 0.25 $\mu\text{m}$ ). The samples were run under the following conditions: injection port temperature: 175°C; flow: 1 mL/min He; 10:1 split ratio; GC oven: 100°C (hold 3 minutes); 10°C/min to 250°C; 250°C (hold 5 minutes); total run time was 23 minutes. The identified additives were primarily nitroglycerin (NG), ethyl centralite (EC), diphenylamine (DPA), and dibutyl phthalate (DBP). Of the 90 analyzed powders, 77% were double base (NG containing). All Winchester and Alliant powders in this study were double base. Seventy-seven% of the samples contained DPA, and 76% contained EC. All powders had at least two additives present at detectable levels. The preliminary results show that EC was found in products from IMR, Hodgdon, Winchester, Alliant, and Vihtvuori. 2,6-dinitrotoluene was identified in products only from Hodgdon and IMR. It was noted that many samples gave the same GC/MS additive profiles but had different powder morphologies; demonstrating the need for the fusion of data from different analytical methods for more robust sample differentiation and comparison. A two-tiered method for combining both data sets (morphology and additive profiling) will be presented. The results from this study are very encouraging but will require more samples to obtain a more robust assessment.

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### Smokeless Powder, Improvised Explosive Device, Image Analysis



### B90 Yes, Everybody Knows a Fire Needs Oxygen, But Why Should We Care?

John J. Lentini, BA\*, *Scientific Fire Analysis, LLC, Islamorada, FL 33036*

**Learning Overview:** After attending this presentation, attendees will: (1) have a greater appreciation for the role of oxygen in the development of fire patterns, (2) understand some of the history of fire investigation with respect to all three legs of the fire triangle, and (3) understand the history of changes in fire investigation techniques.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing results of tests measuring oxygen consumption and providing a greater appreciation for the role of oxygen in the behavior of compartment fires.

“The fire triangle,” fuel, heat, and oxygen is something that fire investigators have known forever. It is grammar school science. However, what is new about the fire triangle is a recent appreciation for the importance of oxygen.

In just about every fire, the source of oxygen is the earth's atmosphere, which contains 20.95% oxygen. The depletion of oxygen by the fire itself is something that has only recently begun to be appreciated. Experiments conducted by the Bureau of Alcohol, Tobacco, and Firearms (ATF) at their National Training Facility and at outside meetings of fire investigators beginning around 2005 revealed that because of oxygen depletion, as well as enhancement around sources of oxygen, fully involved fires can produce fire patterns that tend to mislead investigators into determining the wrong area of origin, something that almost inevitably leads to determining the wrong cause of the fire.

A new focus on considering oxygen when determining the origin of the fire was put forward by Cox in 2013 in a paper entitled “Origin Matrix Analysis.”<sup>1</sup> The conclusion is that once a fire becomes fully involved, patterns produced subsequently offer little or no insight into where the fire started. Utiskul et al performed an elegant experiment showing the movement of flames from the source of the fuel to the source of the oxygen once the compartment becomes saturated with the products of combustion.<sup>2</sup>

More recently, Underwriters Laboratories (UL) Firefighter Safety Research Institute has conducted experiments showing the utter dependence of a fire's temperature on the oxygen concentration. Once a fire reaches the point of flashover, the oxygen concentration drops dramatically, as does the temperature.

Throughout much of the 1980s and 1990s, the fire protection engineering profession tried to educate the fire investigation profession on the importance of radiation in compartment fires. For the most part, that attempt succeeded, and now there are fewer incorrect determinations of fire cause based on “low burning.” After a certain point, burning on the floor means only that the room has become fully involved.

The current task is to acquaint fire investigators with a procedure for considering the effects of ventilation on the production of fire patterns. NFPA 921, *Guide for Fire and Explosion Investigations*, contained, for its first eight editions, a series of five drawings showing the progression of fire growth in a compartment starting with free burning and ending with full room involvement. In the ninth (2017) edition, the diagram showing full room involvement was changed to demonstrate that the only place flames are likely to be found is where there is a source of oxygen, and the patterns produced in those places may have nothing to do with where the fire started.<sup>3</sup>

This presentation will show some of the results from the UL tests, and leave attendees with a greater appreciation for the role of oxygen in the behavior of compartment fires.

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1. Cox (2013), Origin matrix analysis: a systematic methodology for the assessment and interpretation of compartment fire damage, *Fire and Arson Investigator*, 64 (1), 37-47. Crofton, MD, International Association of Arson Investigators.
2. Utiskul, Y., Quintiere, J. G., Rangwala, A. S., Ringwelski, B. A., Wakatsuki, K., Naruse, T. (2005) Compartment fire phenomena under limited ventilation. *Fire Safety Journal*, 40(4):367-390. Elsevier.
3. NFPA (2014), NFPA 921, *Guide for Fire and Explosion Investigations*, Quincy MA, National Fire Protection Association. 45-47.

#### Oxygen, Ventilation, Temperature



### **B91 A Large-Scale Study for the Differentiation of Individuals Based on Triacylglycerols (TG) in Latent Fingerprints**

*Kelly C. O'Neill\*, Iowa State University, Ames, IA 50011; Paige L. Hinners, MS, Ames, IA 50010; Young-Jin Lee, Iowa State University, Ames, IA 50011*

**Learning Overview:** After attending this presentation, attendees will learn how Triacylglycerol (TG) species from latent fingerprints can be used to differentiate between individuals, specifically between people with and without diabetes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic field by providing another potential way to utilize mass spectrometry imaging data for forensic analysis in cases where fingerprints have no match in any database. In the current study, the TG profile is used to gain insight into the health status of the suspect.

**Introduction:** Matrix assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) is being widely researched for use in the forensic field, particularly for the chemical analysis of latent fingerprints. MALDI-MSI is used to study the chemical composition of fingerprints and can provide a visual image in addition to the chemical information. The chemical composition of a fingerprints offers a means to obtain information about the suspect when no database match is available. For this technique to be useful, there must be chemical species that can differentiate individuals. In this work, the authors attempted to use TG species to distinguish between individuals that have diabetes and those that do not. TGs were chosen as a focus for this differentiation because diabetes is a known metabolic disorder and is therefore directly linked to TG levels.

**Methods:** Fingerprint samples were collected from various students, staff, and faculty from Iowa State University. The fingerprints were sprayed with a 10 mM solution of sodium acetate using a TM sprayer (HTX Technologies) and sputter coated for 10 seconds with a gold target. A MALDI-Linear Ion Trap-Orbitrap mass spectrometer was used to collect mass spectra from each of the fingerprints from  $m/z$  500-1000, where TG species are found. Signal intensities were extracted from the spectra for each of the TGs and normalized to the most abundant TG in the fingerprint. Statistical analysis was done using the online software, MetaboAnalyst.

**Preliminary Results:** Thus far, fingerprints have been collected from 33 individuals of varying age, gender, and race. Nine of the 33 fingerprints collected have been from individuals diagnosed with type I or type II diabetes. Using the heatmap feature in MetaboAnalyst, initial patterns in the TG profiles were discovered. The authors began by focusing on only female participants to limit the number of variables in the statistical analysis. The heatmap displayed the TG profile of each female participant in the form of a matrix of colored cells, where the color indicates the value or signal intensity for each of the TG species. Two major clusters were visible in the data, one containing mostly healthy individuals and the other containing mostly participants with diabetes, with a few exceptions. Interestingly, the cluster containing most individuals without diabetes show more abundant TGs with higher levels of unsaturation, whereas the cluster containing mostly diabetic individuals have increased levels of saturated TGs. This finding is consistent with previous research that has shown the release of insulin may be inhibited by saturated long-chain fatty acids.

**Conclusion:** Preliminary results show promise for the possibility of distinguishing individuals by health status, particularly those with diabetes, from healthy individuals based on their TG profiles. Future work will include collecting fingerprints from more individuals to increase the statistical accuracy of the data. We also hope to incorporate a machine learning algorithm into data analysis to predict whether an individual has diabetes based on the TG profile in their fingerprint.

**Individual Differentiation, Latent Fingerprints, Mass Spectrometry Imaging**



### B92 Tape-Dependent Latent Print Development

Vidia A. Gokool, BSc\*, Miami, FL 33157; Monika Garcia, MFS, Defense Forensic Science Center, Forest Park, GA 30297; Rachel L. Creager, Defense Forensic Science Center, Forest Park, GA

**Learning Overview:** After attending this presentation, attendees will better understand which latent print processing techniques are best suited for initial development of latent prints on the adhesive side of a variety of pressure sensitive tape types.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by creating a tape-dependent listing of complementary processing methods which offer a consistent, straightforward approach to developing latent prints upon initial processing of the adhesive side of tape.

As a commonly encountered source of forensic evidence, tape samples are processed for latent prints which may be present on both the non-adhesive backing and the sticky, adhesive side of tape. Improving the quality of latent prints developed on the adhesive side of tape is a necessary endeavor for the advancement of evidence processing.

A comparative study of dye stains, powders in suspension, and one-step fluorescent technique was conducted to determine the optimal pairing of development method and tape sample. The clarity of latent prints produced at three intervals of age were evaluated for development on the adhesive side of duct tape, electrical tapes (black and blue), and cellophane tapes (clear packing, brown packing, and Scotch® Magic™ tape). Nine processing methods (alternate black powder, Basic Yellow 40, gentian/crystal violet, Liqui-Drox, powder in suspension, Rhodamine 6G, Sticky-Side Powder, TapeGlo™, and Wetwop®) were tested on each included tape type and evaluated for quality of print development after samples had been aged for 24 hours, 2 weeks, and 6 weeks.

The evaluation and scaling of each process and tape combination was completed two ways: 1) Processed samples were subjectively analyzed by latent print examiners and rated on a pre-defined 0 to 3 scale for clarity as it pertains to the overall ridge development, as well as the visible presence of Levels I, II and III friction ridge detail; and 2) Processed samples were rated for clarity utilizing the FBI's Latent Quality Metrics (LQMetrics) software. Numerical scalings were objectively assigned in determining the overall quality and clarity of the latent prints produced by the test methods. The overall sensitivity of each processing technique was thus analyzed for quality of developed latent prints and effectiveness of the technique at processing varying qualities of samples.

This presentation will advance the field of latent print examination by providing a list of tape types and complementary processing methods. Tailoring a straightforward approach to each type of tape sample decreases the chances that latent prints will remain undeveloped by processing with a suboptimal method. The decrease in unsuccessful initial processing will also lead to faster turnaround for tape-based casework processing. In whole, this study moves towards structuring the latent print analysis of tapes to increase successful development of latent prints upon first examination through educating examiners on the comparative values of multiple processing techniques in developing latent prints on the adhesive side of a variety of tape types.

*The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.*

*Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, DFSC, U.S. Army Criminal Investigation Command, OPMG, DA or DoD.*

#### Tape, Adhesive, Latent Prints





### **B93 Latent Print Processing of Glassine Stamp Bags Containing Suspected Heroin: The Search for an Efficient and Safe Method**

*Brittany Barnes, BS, Allegheny County Office of the Medical Examiner, Pittsburgh, PA 15222; Jason Clark, MS, Allegheny County Office of the Medical Examiner, Pittsburgh, PA 15222; Joseph B. Kadane, PhD\*, Carnegie Mellon University, Department of Statistics, Pittsburgh, PA 15213; Marla Priestley, BS, Allegheny County Office of the Medical Examiner, Pittsburgh, PA 15222; Neil Spencer, MS, Department of Statistics & Data Science, Pittsburgh, PA 15213; Deborah Tator, MS, Allegheny County Office of the Medical Examiner, Pittsburgh, PA 15222; Denielle Wauthier, BS, Allegheny County Office of the Medical Examiner, Pittsburgh, PA 15222; Joshua Yohannan, MS, Allegheny County of the Medical Examiner, Pittsburgh, PA 15222*

**Learning Overview:** After attending this presentation, attendees will learn how a series of experiments permitted the Allegheny County Medical Office of the Medical Examiner (ACOME) to improve its standard operating procedure (SOP) for searching glassine drug bags for fingerprints and for reporting the weight of power therein.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how analysts are safer because they are less exposed to the unknown contents of drug bags, specifically fentanyl and its analogues, with the new procedure. The procedural change also permits ACOME to respond more quickly to law enforcement requests for fingerprints that might be found on the bags.

At the start of this study, ACOME's SOP required analysts to remove the contents of seized drug bags to a different, pristine bag before analysis for fingerprints could begin. This exposed those analysts to whatever drugs might be in the bags. With the increase of fentanyl and similar drug submissions, it became urgent to reduce that exposure. The reason for removing the drugs was that for legal proceedings, it was important to be able to report the weight of the drug seized. It was thought that fingerprint processing methods, particularly the humidity required to use ninhydrin as an enhancing agent, might cause heroin to convert to monoacetylmorphine (MAM). Hence, the first study reported here is a qualitative analysis to see if processing in the presence of fentanyl destroyed heroin. The results showed that heroin was still present.

The second study was an experiment designed to compare the efficacy of four methods of enhancing fingerprints: magnetic powder, ninhydrin with chamber, 1, 8-Diazalluron-9-One (DFO) with chamber and a sequential treatment of DFO and ninhydrin, both with chamber. Fingerprints on pristine bags were collected from five volunteers; the resulting prints were photographed and evaluated independently by four fingerprint analysts, using a four-point scale. The results suggested that magnetic powder was the most effective method. However, the prints were analyzed only a few days after deposition, which would favor magnetic powder over the other methods that rely, not on water secretion, but on amino acid secretions that are not water soluble. Consequently, this result is held in abeyance, pending the results of a further experiment in which fingerprinted bags are held for up to a year before processing.

The third study is a gravimetric analysis, comparing the weight of the bags before and after analysis. It showed that the average weight increased less than the uncertainty of measurement, and hence is trivial.

The upshot of these studies is that the ACOME SOP is now to use ninhydrin to search for fingerprints on drug bags without emptying them first. Then, if weight of drugs become a legal issue, the weight can be estimated later using standard methods.

#### **Fingerprints, Heroin Bags, Fentanyl**

## B94 Latent Fingermark Detection Using Functional Nanomaterials

Fehmida Kanodarwala, PhD\*, University of Technology Sydney, Sydney, New South Wales 2007, AUSTRALIA; Po Lun Lee, BSc, University of Western Sydney, Sydney 2751, AUSTRALIA; Sebastien Moret, PhD, University of Technology Sydney, Sydney 2007, AUSTRALIA; Xanthe Spindler, PhD, University of Technology Sydney, Sydney 2007, AUSTRALIA; Christopher J. Lennard, PhD, University of Western Sydney, Richmond, NSW 2753, AUSTRALIA; Claude Roux, PhD, University of Technology Sydney, Broadway, NSW 2007, AUSTRALIA

**Learning Overview:** The goal of the presentation is to provide an outlook on the use of nanoparticles for the detection of latent fingermarks.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the possibility of engineering nanoparticles for latent fingermark detection and thus achieve success in using them for routine forensic examinations such as fingermark detection.

Nanoparticles (NPs) have been used in several applications like solar cells, drug delivery, LEDs etc. Application for fingermark detection has been explored mainly due to their small size, functionalization of their surface, and luminescence properties. Despite all the research that has been done so far on the use of nanoparticles for fingermark detection, few publications present clear supporting evidence of their superiority over commonly used techniques. The problem is that nanoparticles are often designed for applications outside forensic science and are then tentatively applied onto fingermarks with limited success.

Significant research has been conducted on nanoparticles for fingermark detection applications as a novel approach that promises increased sensitivity and selectivity. Different kinds of nanopowders such as gold, silver, titanium dioxide, and aluminum oxide have been applied instead of conventional fingerprint powder for fingermark detection.<sup>1</sup> Multimetal deposition (MMD) using silver and gold nanoparticles has been investigated for its application on various surfaces—porous and nonporous, wet and dry, single-metal deposition (SMD)—a simpler alternative to MMD—involving gold enhancement of the deposited gold nanoparticles.<sup>2,3</sup> Different metal oxides including titanium dioxide, aluminum oxide, or zinc oxide often functionalized with aliphatic chains have been reported. Cadmium telluride (CdTe) quantum dots (QDs) have been studied for the detection of weak fingermarks in blood on non-porous surfaces.<sup>4</sup>

No nanoparticle-based approach applied to-date has demonstrated all three advantages (size, functionalization, and optical properties). Silicon oxide nanoparticles appear to be the best candidate to address this issue. They consist of a porous matrix of siloxane bonds with an external layer of silanol groups that can further react through hydrolysis and condensation with various alkoxy silanes to be linked with functional groups; antibodies or aptamers can be grafted onto the NP's surface to selectively target fingermark components.<sup>5</sup> Luminescent dye molecules can be trapped within the siloxane matrix, which provides extended optical properties to maximize contrast between fingermarks and the background. This study presents various syntheses of silicon oxide (SiO<sub>2</sub>) nanoparticles via reverse microemulsion and their subsequent application to detect fingermarks on non-porous substrates. The main goal is to design a technique that is user-friendly, cost-effective, and that can be implemented in routine practice by law enforcement authorities.

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### Fingermark, Nanoparticle, Silicon Oxide



### B95 Why Do Latent Print Examiners Differ in Their Conclusions?

Bradford Ulery, MS, Reston, VA 20191; R. Austin Hicklin, PhD\*, Noblis, Reston, VA 20191; Tom Busey, PhD, Bloomington, IN 47405; Maria A. Roberts, Quantico, VA 22135; JoAnn Buscaglia, PhD\*, FBI Laboratory, CFSRU, Quantico, VA 22135

**Learning Overview:** After attending this presentation, attendees will understand the factors associated with differences in conclusions made by professional latent print examiners.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing the underlying reasons for differences in forensic latent print examiners' conclusions.

Why do latent print examiners reach different conclusions? Previous studies reported reproducibility of conclusions was generally 80-90%, depending on the measure—high but certainly not unanimous—but when examiners described a comparison as “difficult,” reproducibility was 50-55%.<sup>1,2</sup> This work focuses on explaining why latent print examiners do not always reproduce each other's conclusions, particularly focusing on understanding “missed IDs”; why some examiners are inconclusive or make erroneous exclusions on comparisons of mated images when other examiners make identification conclusions. Results are based on an experiment in which eye-tracking technology was used to measure eye-gaze behavior of 121 practicing latent print examiners as they performed over 2000 fingerprint comparisons, along with new analyses of the data from our earlier Black Box and White Box studies.<sup>1-3</sup>

This presentation describes a model of disagreements that quantifies the contributions of different types of disagreements to overall reproducibility and explains how factors such as image characteristics, subjective sufficiency thresholds, procedural errors, and examiner uncertainty contribute to specific decisions. This model offers some predictive utility for recognizing when these factors may affect reproducibility and may be used in addressing these issues in training.

Many differences in conclusions can be explained by individual examiners' tendencies toward specific conclusions, most notably in making decisions of Value vs No Value, Identification vs Inconclusive, and Exclusion vs Inconclusive. These tendencies can be understood as an implicit individual decision threshold that can vary notably among examiners. Most of the remaining differences in conclusions occur close to these implicit individual decision thresholds and are conclusions that are often not repeated by the examiners themselves. The time that examiners spend in analysis and comparison, and the level of difficulty they assess for a comparison, are associated with these implicit thresholds. When examiners are making comparisons close to their implicit individual decision thresholds, the comparisons are generally slow and more likely to be rated difficult.

Eye tracking was used to show how examiners' eye behavior can explain differences in conclusions. When examiners missed IDs (especially erroneous exclusions), they often did not extensively compare the prints under proper alignment. The results provide additional information on the prevalence and reproducibility of erroneous identification and exclusion conclusions (false positives and false negatives). The study shows notable differences among examiners, which has implications for staffing, assigning verifiers, and disagreements by experts in court; examiner-specific performance rates could be measured in rigorous proficiency testing like a black box test and used operationally.

**Hypothesis statement:** The differences in conclusions made by forensic examiners can be explained (at least in part) by eye gaze behavior and other factors.

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#### Latent Prints, Examiner Behavior, Eye Tracking



### **B96 Assessing the Expected Weight of Evidence for a Latent Print (Fingermark) That Is Insufficient for Identification and Without Reference to a Putative Source**

*Marco De Donno\**, Batochime, University of Lausanne, Lausanne, Switzerland, SWITZERLAND; *Christophe Champod*, PhD, University of Lausanne, Lausanne-Dorigny, Vaud 1015, SWITZERLAND; *David A. Stoney*, PhD, Stoney Forensic, Inc, Chantilly, VA 20151; *Paul Stoney*, MBA, Stoney Forensic, Inc., Clifton, VA 20124

**Learning Overview:** After attending this presentation, attendees will understand a method to assess the expected weight of evidence for latent prints (fingermarks) that are insufficient for identification, without reference to a putative source.

**Impact on the Forensic Science Community:** This presentation will impact the scientific community by providing a means to assess the expected weight of evidence for latent prints (fingermarks) that are currently set aside as insufficient for identification.

Latent prints that have insufficient characteristics for identification often have discernable characteristics that could form the basis for lesser degrees of correspondence or probability of occurrence within a population. Currently, those latent prints that experts judge to be insufficient for identification are not used as associative evidence.

The aim of this presentation is to expose the methodology developed to assess the value of a fingermark without its putative source. By “value” means an assessment of the expected weight of evidence (WoE quantified by a Likelihood Ratio or Bayes factor) that a mark may bring if it was compared with it corresponding print. Because the proposed assessment of the Weight of Evidence is made without the corresponding print, it is called a *pre-assessment*, based on the sole merit of the features of the mark.

The proposed methodology is based upon the model of Egli (2009) enhanced by a distortion model that produces a score-based Likelihood Ratio.

When used in regular casework (hence following a comparison between a mark and a print), the score-based Likelihood Ratio system requires minutiae information from both the mark and the print. In the pre-assessment mode adopted here, the print is not available and will then be postulated. To do so, one will assume that the potential print that should be associated with the mark presents the same minutiae configuration (in terms of x-y positions and angles theta) as the minutiae on the mark. The expected score-based Likelihood Ratio is then calculated between the mark and the mark itself. Based on the score obtained from that transaction, a model is developed to estimate the expected score-based Likelihood Ratio (ESLR) if the putative reference would be available. The model is calibrated against the score-based Likelihood Ratio obtained in known cases where marks and corresponding prints are available.

This project has shown that it is possible to predict the expected value (ESLR) to be assigned to a mark solely based on the features of available on the mark. The closer prediction to the target score-based Likelihood Ratio are achieved when an expert manually annotates the minutiae on the mark. However, reasonable prediction is achieved when the mark is auto-encoded using a latest generation Automatic Fingerprint Identification System (AFIS) minutiae encoder and the minutiae are further selected as a function of quality metric exported by the AFIS system. A fully automatic system for pre-assessing marks is proposed and has been calibrated using a linear regression model.

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#### **Fingerprint, Weight of Evidence Pre-Assessment, AFIS**



### **B97 The Associative Value of Latent Print Correspondences That Are Insufficient for Identification**

*David A. Stoney, PhD\*, Stoney Forensic, Inc, Chantilly, VA 20151; Marco De Donno, Batochime, University of Lausanne, Lausanne, Switzerland, SWITZERLAND; Christophe Champod, PhD, University of Lausanne, Lausanne-Dorigny, Vaud 1015, SWITZERLAND; Paul Stoney, MBA, Stoney Forensic, Inc., Clifton, VA 20124*

**Learning Overview:** After attending this presentation, attendees will understand that there are many latent prints that have high associative value but lack sufficient value for identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing new insight into the possibilities of exploiting latent prints that are currently set aside as "of no value for identification."

Currently, those latent prints that experts judge to be insufficient for identification are not used as associative evidence. How often do such prints occur? What is their potential value for association? Would they impact case investigations or prosecutions in a useful way?

Latent prints, previously determined to be of no value for identification (NVID Latents) were collected from six principal donor laboratories. A total of 1715 photographs and latent lift images were collected from 1026 cases representing 823 property crimes (19.8%) and 203 violent crimes (80.2%). Administrative screening and cropping of these images resulted in 1408 latent prints from 805 cases. To address variability in NVID decisions among laboratories and examiners, and ensure that the prints met program requirements, each latent print was re-examined by a single, highly qualified, certified latent fingerprint examiner (Pat Wertheim). Program requirements were latent prints with discernable Level 2 ridge detail and 3 or more Level 2 ridge characteristics, but without sufficient ridge detail for identification. These requirements were met by 974 NVID latent prints representing 595 cases.

Measurements of associative value were made using an expected score-based likelihood ratio (ESLR). Auto-encoding of minutiae was performed using a SAGEM-Morpho Light-Out system in version 10. Similarity scores were computed using a Morpho DMA equipped with a matcher in version 9, based on minutiae meeting a quality level of 11 or above (using a scale from 2 to 14 as defined by the Light-Out minutiae detector).

A total of 661 NVID Latent prints showed a  $\text{Log}_{10}$  ESLR values between 2 and 10.9, with a mean of 5.7 (a likelihood ratio expected from a frequency of occurrence of about 1 in 500,000).

The actual usefulness of these prints depends on the case context. Work is continuing with investigators, prosecutors and crime laboratories to study prints found in well-defined case contexts. This approach is designed to answer whether, how often and to what degree associations from these latent prints could answer questions of relevance within specific case contexts.

*This project was supported in part by Award No. 2016-R2-CX-0060 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.*

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#### **Fingerprints, No Value For identification, Associative Value**



### **B98 Probabilistic Reporting in American Criminal Cases: A Baseline Study**

*Simon Cole, PhD\*, University of California, Irvine, CA 92697-7080; Matt Barno, JD, University of California, Irvine, CA 92697-7080*

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**Learning Overview:** After attending this presentation, attendees will gain an empirically grounded understanding of the current state of probabilistic reporting in six criminalistic disciplines in the United States.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping forensic scientists understand empirically how forensic results are reported in American trials today. The reporting of forensic results is a topic of crucial importance and increasing interest. Given that many forensic statisticians are advocating for the greater use of probabilistic reporting, this research may allow them to establish a baseline in order to measure progress toward that goal.

Over eight years ago the NRC Report, *Strengthening Forensic Science in the United States*, highlighted the lack of standards regarding reporting of evidence in forensic science. At the same time, forensic statisticians have been calling for the greater use of probabilistic reporting in forensic science. Yet, despite the intense interest surrounding forensic evidence reporting, there have been few efforts to empirically survey how forensic examiners are currently reporting evidence in ordinary, everyday criminal cases. Previous studies of forensic evidence reporting primarily have relied on either official reporting standards or anecdotal data from high-profile cases, neither of which are likely to yield a representative view of actual reporting. By contrast, the current study seeks to provide insight into how more typical forensic examiners are reporting results in average, run-of-the-mill criminal cases. This study addresses this question for six forensic pattern recognition disciplines: latent prints, firearms and tool marks, questioned documents, shoeprints, tire tracks and blood pattern analysis.

Collecting data on actual forensic evidence reporting is difficult. There is no comprehensive or representative repository of forensic expert reports and testimony. Accordingly, the current study relies on an opportunistic sample of forensic expert reports and testimony culled from a variety of sources. Based on trial transcripts, affidavits, laboratory reports, and proficiency test submissions, the study measures the degree to which probabilistic reporting occurs. For probabilistic reports, the study describes the type of probability used. Broadly, the probabilistic reporting in these six disciplines is rare. The probabilistic reporting that does exist tends to refer to verbal characterizations rather than quantitative statements based on formal data. The current study will contribute to the ongoing normative discussion among academics and statisticians regarding how forensic results could and should be reported.

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#### **Reports, Statistics, Probability**



### **B99 A Pathway Toward Firearm Population Statistics**

*Xiaoyu A. Zheng, MS\*, National Institute of Standards and Technology, Gaithersburg, MD 20899; Erich D. Smith, MS\*, Federal Bureau of Investigation, Quantico, VA 22135; Martin Baiker-Sørensen, PhD, Netherland Forensic Institute, The Hague, Zuid-Holland 2497 GB, NETHERLANDS; Johannes A. Soons, PhD, National Institute of Standards and Technology, Gaithersburg, MD 20899*

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**Learning Overview:** After attending this presentation, attendees will know about a project to develop a reference tool mark population database and associated statistical distributions of similarity scores to quantify the weight of evidence of a comparison. The presentation will highlight the overall design, workflow, potential implementation steps, and limitations of the system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing a foundational statistical resource for reporting statistical confidence in firearm and tool mark examination.

The database is intended to serve as a foundational resource for tool mark examiners to report a statistically sound estimate for the weight of the evidence or uncertainty of an examination result. The population statistics describe the frequency distributions of a similarity score for, respectively, same-source comparisons and different-source comparisons of tool marks, such as firearm marks on cartridge cases and bullets. Like DNA analysis, these distributions are needed for the estimation of the weight of the evidence, such as a likelihood ratio, or other confidence metrics such as error rates. The effort builds on the NFI Scratch software, which provides a framework to create and maintain a reference tool mark database and associated statistical analysis tools. The database will contain measurement data of tool marks from a large variety of known tools, including the Federal Bureau of Investigation (FBI) firearm reference collection. The software mines the database for tool mark comparisons of a population relevant to a case and generates the respective frequency distributions. These distributions can then be used to evaluate the weight of the evidence. The system is designed to enable the use of different similarity metrics or scores, such as those developed by the National Institute of Standards and Technology (NIST) and the Netherlands Forensic Institute (NFI). The system is an important component in ongoing efforts by the FBI to implement objective measurement and analysis into casework. The presentation will highlight the overall design, workflow, potential implementation steps and limitations of the system.

No matter what statistical method or model is used, a large and diverse reference collection of ground truth tool mark data is required to achieve meaningful results. Therefore, the framework is designed as a living system to be maintained and updated on an ongoing basis with new tool marks and comparison metrics to ensure relevance to actual casework. The resulting reference populations can also be used to further advance research on objective comparison metrics and statistics, allowing developers to provide better tools to support the examiner's conclusions. There is also potential for this research to be applied in other pattern evidence disciplines.

*The project was a joint effort by the National Institute of Standards and Technology (NIST), the Federal Bureau of Investigation (FBI), and the Netherlands Forensic Institute (NFI).*

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### **Firearms, Statistics, Populations**



### B100 An Experiment to Explore Persistence of Bullet Striations

Heike Hofmann, PhD\*, Center for Statistics and Applications in Forensics, Ames, IA 50011; Alicia L. Carriquiry, PhD, Center for Statistics and Applications in Forensics, Ames, IA 50011; Nicholas A. Lennie, BSc, Story County Sheriff's Office, Iowa, Nevada, IA 50201

**Learning Overview:** After attending this presentation, attendees will better understand study design, 3D imaging, and persistence of bullet striations. Attendees will also learn about new technologies for comparing bullets using 3D images of land impressions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by presenting experimental data that permit exploring the fundamental assumption of repeatability of striations on bullets.

Firearms examination relies on two fundamental assumptions—uniqueness and repeatability. The uniqueness assumption states that every gun leaves a unique pattern of striations on bullets and cartridge cases. The repeatability assumption states that these unique marks do not change over time unless the inside of the barrel of the gun are altered by mechanical or chemical means. If striations are repeatable, it is said that the markings are *persistent*.

To better understand the concept of persistence, researchers in the Center for Statistics and Applications in Forensic Evidence, in collaboration with the Story County Sheriff's Office in the State of Iowa and the Division of Criminal Investigation in Iowa conducted a controlled experiment to collect information useful to explore the question of persistence. The timing for the study was fortuitous, since early in the year the Story County Sheriff's Office decided to switch to Sig Sauer to use as the official firearm for all law enforcement personnel. Consequently, over 20 brand new Sig Sauer P320 pistols were made available to us to conduct the study. Data collection is still ongoing, but the first set of 20,000 test fires has already been collected and imaged. In a second phase of the study, 15 Smith and Wesson pistols confiscated from suspects and provided by the DCI are being used to replicate the study.

Both the bullet and the cartridge case were collected from each test shot included in the study. To collect the samples, guns were fired into a cylindrical steel tube packed with Kevlar fibers and equipped with three side doors to facilitate finding the bullet. As the guns had never been fired, the first 10 test shots were of interest and were kept for imaging. After the first ten shots, the first three bullets and cartridge cases out of every fifty were collected for imaging. Therefore, for each gun the ammunition that was set aside for imaging included shots number 1-10, 50-52, 100-102, 150-152 and so on through 2000-2002. The Sig Sauer P320 magazine holds 17 rounds, so between each set of 3 experiment shots, almost three full magazines were emptied into the berm surrounding the firing range of the Story County Sheriff's Office. Every gun was taken apart and both the inside of the barrel and the breech face were cleaned with gun oil and soft brushes and cloth every 50 shots. Guns had a chance to cool down between being shot 50 consecutive times.

The working hypothesis is that a barrel produces different patterns of striations until it "settles." If so, then bullets 50-52 would be less like bullets 100-102 than bullets 1500-1502 are to 1600-1602. Related questions of interest are: (1) When do the Sig Sauer barrels settle (i.e., when do markings begin to exhibit persistence), (2) What is the variability in terms of time to persistence across similar guns, and (3) What differences will be seen when the shots are from the set of Smith and Wessons with different level of use and care?

In this presentation, only results obtained from the Sig Sauer guns will be discussed. The data for analysis are the 3D images of land impressions, and the comparisons between pairs of bullets are carried out using the algorithms developed by CSAFE researchers in Iowa State University.<sup>1,2</sup> The persistence dataset is a valuable resource for researchers in this area and will be placed in the public domain as soon as possible.

#### Reference(s):

- <sup>1.</sup> Hare E., Hofmann H., Carriquiry A.: Algorithmic Approaches to Match Degraded Land Impressions., Law, Probability and Risk, accepted, doi:10.1093/lpr/mgx018.
- <sup>2.</sup> Hare E., Hofmann H., Carriquiry A.: Automatic Matching of Bullet Lands., Annals of Applied Statistics, accepted, doi: 10.1214/17-AOAS1080.

#### Bullet Striations, Persistence, 3D Imaging





### **B101 Comparison of VisionX and Leica® UFM4 Comparison Microscopes and Validation of the VisionX Comparison Microscope for Intra- and Inter-Laboratory Examination**

*Mark S. Dreyfuss, PhD\*, Maryland State Police, Forensic Sciences Division, Pikesville, MD 21208; Daniel E. Katz, MFS, Maryland State Police, Forensic Sciences Division, Pikesville, MD 21208; Torin Z. Suber, Maryland State Police, Forensic Sciences Division, Pikesville, MD 21208; Susan L. Kim, MFS, Maryland State Police, Forensic Sciences Division, Pikesville, MD 21208*

**Learning Overview:** After attending this presentation, attendees will understand the technical advances in the forensic examination of firearms and tool marks and how newly available technology can be used to expand peer to peer communications and improve the decision-making process during the microscopic comparisons of bullets, cartridge cases and tool marks. Results of an independent validation study of two comparison microscopes will question whether current requirements to perform same location/same scope verifications are necessary. Results evaluating the VisionX for intra- and inter-laboratory examinations will also be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing whether the principles of the firearms and tool marks community require updating considering new comparison microscopes enhancements and communication technology.

The VisionX comparison microscope integrates advanced computer technology for the microscopic comparison of bullets, cartridge cases, and tool marks. Additional capabilities of the VisionX software allows for enhanced peer-to-peer communication and statistical analysis during the examiner's decision-making process. Validation of new equipment in a forensic laboratory requires not only that the equipment perform as expected, but also that it can produce results that are useful for law enforcement and acceptable in court settings. To accomplish a validation of the VisionX comparison microscope, a series of projects were designed and implemented.

Project 1 compared the VisionX comparison microscope with the Leica UFM4 comparison microscope by evaluating each microscope's ability to provide measurements of land and groove impressions and highlight characteristics of fired bullets that are required for entry into the Federal Bureau of Investigation (FBI) General Rifling Characteristics (GRC) database. Samples were gathered from handguns submitted to the Maryland Handgun Roster Board (HRB) as part of the approval process to sell a new handgun in Maryland.

Project 2 functioned as an in-place verification that the new VisionX technology and currently used Leica UFM4 are, at a minimum, equivalent in function for firearms casework. Four firearms examiners were given standardized proficiency examinations that required the identification of matched and unmatched .40 S&W cartridge cases fired from consecutively manufactured slides for a Smith and Wesson model SW40VE. Each examiner was tested for accuracy and time of determination and surveyed for their impressions of the two comparison microscopes.

Project 3 was designed to evaluate the need for on-site examiners who visually inspect all cartridge casings to be present in the same location. By linking two VisionX Comparison Microscope Stations or by linking a VisionX Comparison Microscope Station to an examiner's office computer, examiners within a single lab can see live camera images at the same time but not in the same location or at the same microscope. The ability of pairs of examiners to share the same live camera image and discuss or text each other in real time so that independent determinations can be made based on the same agreed-upon image will be discussed.

Project 4 expands the peer to peer connectivity tests by having Maryland State Police firearms examiners share images with other firearms examiners located in other counties, states, and countries.

Last, Project 5 applies the capabilities of the VisionX software to create data that can be used to calculate a statistical relevance value for the comparison conclusions rendered during the validation study.

*\*Supported by a grant from the National Institute of Justice (2016-DN-BX-K006)*

### **Firearms and Tool Marks, Comparison Microscopes, Inter-Laboratory Communication**



## B102 The Development and Validation of Machine Learning Models for Fire Debris Analysis

Michael E. Sigman, PhD\*, University of Central Florida, Orlando, FL 32816; Mary R. Williams, MS, National Center for Forensic Science, Orlando, FL 32816-2367

**Learning Overview:** After attending this presentation, attendees will understand about machine learning methods and how these methods can be validated and applied in fire debris analysis.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by demonstrating that data analysis methods based on machine learning can objectively evaluate fire debris data and render a probabilistic estimate of the evidentiary value, which is not possible utilizing current methods based on ASTM E1618-14.

Machine learning is an artificial intelligence method that relies on statistical techniques to learn complex data patterns and make predictions on new data. Real-world applications of machine learning include voice recognition systems on cell phones, internet search engines, facial recognition, and traffic pattern predictions, among many others. Complex problems in forensic science are also amenable to machine learning applications. One example application of a challenging data analysis problem in forensic science is the detection of ignitable liquid residue in fire debris data. Several processes contribute to the complexity of recognizing the presence of an ignitable liquid residue in fire debris. Partial combustion and pyrolysis of substrate materials in a fire produce many chemical compounds that are also found in commercial ignitable liquids. Fire often leads to partial evaporation of the ignitable liquid, resulting in a modified chromatographic profile. Highly varied background signals and biological degradation effects combine to further-enhance the data analysis challenge.

Unlike currently used methods of data analysis that rely on the analyst for visual pattern recognition, machine learning methods are not subjective and can produce either categorical decisions or validated estimates of evidential value in the form of likelihood ratios. Machine learning methods, such as support vector machines, quadratic and linear discriminant analysis, k-nearest neighbors, and others have been used in research to estimate the evidentiary value of fire debris samples, even in the presence of highly varied background signals.<sup>1-5</sup> Research results from the use of machine learning in the analysis of fire debris data will be presented. Validation of machine learning results will be evaluated based on performance metrics, including receiver operating characteristic analysis, detection error tradeoff plots, Tippett plots and empirical cross entropy plots.<sup>4,5</sup>

Similar validation metrics are not available for currently used fire debris data analysis methods based on ASTM E1618-14. Calculation and reporting of likelihood ratios based on the machine learning methods will be discussed.

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2. (a) Waddell, E.E.; Song, E.T.; Rinke, C.N.; Williams, M.R.; Sigman, M.E. Progress toward the determination of correct classification rates in fire debris analysis. *Journal of Forensic Sciences* 2013, 58, 887-896. (b) Waddell, E.E.; Williams, M.R.; Sigman, M.E. Progress toward the determination of correct classification rates in fire debris analysis ii: Utilizing soft independent modeling of class analogy (simca). *Journal of Forensic Sciences* 2014, 59, 927-935.
3. Lopatka, M.; Sigman, M.E.; Sjerps, M.J.; Williams, M.R.; Vivo-Truyols, G. Class-conditional feature modeling for ignitable liquid classification with substantial substrate contribution in fire debris analysis. *Forensic Sci Int* 2015, 252, 177-186.
4. Sigman, M.E.; Williams, M.R. Assessing evidentiary value in fire debris analysis by chemometric and likelihood ratio approaches. *Forensic Sci Int* 2016, 264, 113-121.
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### Fire Debris, Machine Learning, Method Validation



### **B103 The Detection and Forensic Analysis of Trace Fuel-Oxidizer Mixture Evidence by Infrared Thermal Desorption (IRTD) With Direct Analysis in Real-Time Mass Spectrometry (DART®-MS): Black Powders and Black Powder Substitutes**

*Thomas P. Forbes, PhD\**, National Institute of Standards and Technology, Gaithersburg, MD 20899; *Jennifer R. Verkouteren, MS*, Gaithersburg, MD 20899; *Edward Sisco, PhD*, National Institute of Standards and Technology, Gaithersburg, MD 20899; *Matthew E. Staymates, MS*, National Institute of Standards and Technology, Gaithersburg, MD 20899

**Learning Overview:** After attending this presentation, attendees will understand how coupling Infrared Thermal Desorption (IRTD) with Direct Analysis in Real Time Mass Spectrometry (DART®-MS) can be utilized to analyze and differentiate trace collections of fuel-oxidizer mixtures, specifically targeting black powder and black powder substitutes. Attendees will be presented with representative chemical signatures for these compounds at multiple points in the desorption profile, as well as their identification and discrimination based on multivariate statistics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a unique analytical platform for the trace detection and forensic analysis of wipe-based sample collections, including both organic and nonvolatile inorganic species. The IRTD-DART-MS platform generates rapid and discrete heating ramps, allowing for near simultaneous detection of volatile organic species at lower temperatures and nonvolatile inorganic oxidizers at elevated temperatures. By combining the ability to rapidly identify the full range of chemical species in conjunction with multivariate statistics, this platform provides the forensic examiner a tool to generate high fidelity data for identification and differentiation of black powders and black powder substitutes.

The detection and accurate identification of trace evidence pertaining to homemade explosives remains imperative to the public safety and forensic science communities. Wipe-based sampling of surfaces remains a common method for the collection of trace evidence for further analysis. These sample collections can be chemically analyzed using a wide range of analytical techniques from *in situ* colorimetric detection to laboratory-based liquid chromatography mass spectrometry (LC/MS). Many existing techniques are hindered by targeting a single species or the need for extractions and lengthy analysis times. To combat these constraints, several platforms exist or have recently been developed and/or modified to enable the thermal desorption of target species directly from the wipe-based collection. However, these platforms often maintain a constant thermal desorption temperature, optimized for a specific target analyte or class of analytes. This aspect leads to difficulties in the detection and analysis of nonvolatile inorganic oxidizers found in fuel-oxidizer and self-initiating mixtures commonly playing an important role in homemade explosives as well as illegal or counterfeit fireworks.

To address these hurdles, an infrared thermal desorption (IRTD) platform was developed and coupled with the ambient mass spectrometry technique, direct analysis in real time (DART)-MS, through an enclosed T-junction interface. This coupling takes advantage of traditional ambient mass spectrometry benefits, including, rapid analysis at atmospheric pressure and no additional sample preparation, while introducing a novel mode for analyte thermal desorption. The IRTD platform enabled discrete and rapid (5s to 15s) heating ramps that reached the high temperatures (450°C to 550°C) needed for the thermal desorption of nonvolatile inorganic oxidizer species. The inherent temperature profile generated by the infrared emission allowed more volatile organic species to be thermally desorbed at lower temperatures without degradation or decomposition, followed by the thermally desorption of refractory inorganic oxidizers at elevated temperatures. IRTD-DART-MS demonstrated nanogram to sub-nanogram sensitivities for several common organic explosives and inorganic oxidizers as neat samples. The methodology developed for the detection of these nonvolatile inorganic oxidizers was deployed for the identification and differentiation of a range of black powders, black powder substitutes, and flash powders. These powders represent a class of fuel-oxidizer mixtures of forensic interest due to their abundance and availability as a low explosive or incendiary compound. Due to the diverse ion distributions generated by these complex mixtures, the multivariate statistical method, principal component analysis was employed for their identification and discrimination. In addition, the characteristic temperature programmed desorption achieved by the inherent temperature ramp was exploited for a level of separation of volatile and nonvolatile species. The IRTD-DART-MS and multivariate statistics demonstrated a unique platform enabling the direct identification and differentiation of trace fuel-oxidizer mixtures.

#### **Infrared Thermal Desorption, DART®-MS, Black Powder Substitutes**



### **B104 An Analysis of High Explosive Compounds Utilizing Gas Chromatography (GC) With Tandem Cold Electron Ionization (EI) Mass Spectrometric Detection and Vacuum Ultraviolet Detection (VUV)**

*Dennise Montero, BS\*, Arlington, VA 22203; Ioan Marginean, PhD, The George Washington University, Washington, DC 20007*

**Learning Overview:** After attending this presentation, attendees will better understand the advantages of using combined Mass Spectrometry (MS) and Vacuum Ultraviolet (VUV) detection following the Gas Chromatography (GC) separation of high explosive compounds.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing an alternative analysis method that enhances the molecular ion of the explosive compound and provides an ultraviolet spectrum to improve confidence in identification.

High explosives are characterized by rapid decomposition and development of high pressures which result in detonation with reaction fronts faster than 1000 m/s. Nitrated organic compounds such as trinitrotoluene (TNT) or unstable peroxide compounds such as triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD) are explosives commonly found in bombs. Due to the increasing number of terrorist attacks, explosive identification has become of utmost importance in forensic applications. Most explosive compounds have high thermal instability and require high sensitivity analysis, making their identification a challenging task. Additionally, the variety of explosive groups demands a broad-based analytical technique that is effective with all explosives classes.

Although GC/MS is the most commonly used instrument in forensic analysis, it has certain limitations in the analysis of thermally labile or low volatility compounds (such as high explosives). Traditional GC/MS utilizes classical electron ionization (EI) which may result in excessive fragmentation with little to no molecular ions in the case of labile analytes. Due to absence or very low intensity of the molecular ions in traditional GC/MS with classical EI, explosives are usually identified by their fragmentation pattern. Cold EI often enhances the relative intensity of the molecular ion leading to improved confidence in identification. Survival of molecular ion in the ion source is ensured by intra molecular vibrational cooling of the sample through expansion of supersonic molecular beams prior to their ionization.

In addition to enhanced molecular ion intensities, explosive identification can be further improved through the addition of another analytical dimension such as VUV spectroscopy. Interaction between VUV light and analytes in the gas phase creates unique spectral signatures specific to the chemical structure. The high energy, short wavelength VUV photons probe electronic transitions in the chemical bonds, including ground state to excited state  $\sigma \rightarrow \sigma^*$  and  $n \rightarrow n^*$ . Additionally, the unique spectra allow explosive structural isomers to be clearly differentiated, thus increasing the confidence of identification.

Analysis of high explosives was studied by a cold EI-GC/MS augmented with a VUV detector. Coupling of GC to MS and VUV detectors allows the explosives to be detected by multiple platforms in one run. Various GC parameters (e.g., injection port temperature, ramp rate, and flow rate) were optimized for explosive identification by GC/MS with both classical and cold EI. In most cases, larger relative intensities for molecular ions were observed for explosives analyzed with GC/MS with cold-EI. Certain explosives (for example HMTD) could not be detected in GC/MS with a He flow rate of 1 mL/min. Increasing the He flow rate to 5 mL/min (which is possible in the cold-EI instrument) allows the detection of this analyte. Unique VUV spectra for each explosive were measured and added to the VUV library for future identification. Explosive isomers, such as 2,3-, 2,4-, 2,6-, 3,4, 3,5-dinitrotoluene (DNT), differ only by the position of the two nitro groups on the benzene ring and are difficult to distinguish by GC/MS. However, distinct VUV spectra were obtained, allowing for the isomers to be identified. Overall, the combination of a cold EI-GC/MS with a VUV detector improved the confidence in explosive identification by enhancing molecular ion intensities and providing complementary VUV spectra.

#### **Cold Electron Ionization, Explosives, Vacuum Ultraviolet**



### B105 Forensic Genetic Genealogy for Law Enforcement

*Ellen M. Greytak, PhD\*, Parabon NanoLabs, Inc, Reston, VA 20190; CeCe Moore, Parabon NanoLabs, Reston, VA 20190; Steven Armentrout, PhD, Parabon NanoLabs, Inc., Reston, VA 20190*

**Learning Overview:** After attending this presentation, attendees will understand the science of genetic genealogy and how it can be applied to forensic samples to aid in law enforcement cases. The entire process, from evidence to conclusion, will be discussed, including how the data is generated, how searches are performed, and how genealogy research is used to narrow down the unknown individual's possible identities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the general level of knowledge about genetic genealogy, which is actively being used to solve decades-old cold cases. Attendees will learn about this new technology's investigative potential by seeing how it is used on simulated case studies modeled from actual casework.

Genetic genealogy (GG) is the attempt to identify an unknown individual by finding genetic relatives using a public genetic genealogy database and combining the results with traditional genealogical research of public records. Originally developed to help individuals who have lost their biological identities (e.g., adoptees), the same techniques are now generating leads for law enforcement. GG is distinct from familial searching of law enforcement DNA databases in terms of the type of genetic data used, the source of the comparison samples, and the identification process.

Unlike traditional STR analysis, genetic genealogy begins with the generation of dense autosomal single nucleotide polymorphism (SNP) data from a forensic DNA sample using SNP microarray genotyping or whole-genome sequencing. The Y-chromosome and/or mitochondrial DNA can also be analyzed to determine the individual's haplogroups. GG cannot use a short tandem repeat (STR) profile, as significantly more genetic information is needed to detect distant relatives when comparing data from multiple samples.

The SNP data is uploaded to a public GG database that allows law enforcement use, such as GEDmatch. Software algorithms compare the uploaded data to that from everyone in the database who has allowed such comparisons, reporting back the total amount of shared DNA (in centimorgans, cM) in the autosome and on the X-chromosome. The amount of shared autosomal DNA between two individuals correlates strongly with the degree of biological relatedness. However, for a given degree of relatedness, the amount of DNA sharing observed varies and, moreover, high levels of background relatedness in endogamous populations can inflate observed cM values.

The genetic relatives ("matches") found in the database serve as clues from which traditional genealogy research can proceed. First, each closely matching individual (typically, 3<sup>rd</sup> cousins or closer) must be identified and their family tree constructed using public records, going back to all possible common ancestors with the unidentified person. Once the possible common ancestors have been identified, descendency research is performed to identify all their descendants at the appropriate genetic distance to the match. Finally, the possible identity of the unknown individual must be narrowed down using a variety of sources of information, including triangulation among matches, the unknown individual's known sex and possible age, the geographical location of the crime, and/or predictions of ancestry and phenotype. Depending on the distance and the number of matches, GG can narrow the possible identities of the unknown individual to one, a few, or many. These leads are then investigated by law enforcement, and final identification is performed using direct STR matching.

Simulated case studies will be used to demonstrate how genetic genealogy is used in forensic investigations and the kind of information it can generate. The presenters have evaluated hundreds of forensic samples for their potential to be solved using GG, and statistics on the expected resolution rate for different types of samples will be given. This presentation will provide attendees with sufficient information about genetic genealogy to explain the technology to their agency and determine which cases would be good candidates for GG analysis. All attendees will gain the ability to thoroughly evaluate the accuracy of the many journal articles and media pieces published about GG.

#### Genetic Genealogy, DNA, Single Nucleotide Polymorphism



### **B106 The Characterization and Repair of Hydrolytically Induced DNA Damage in the Mitochondrial DNA (mtDNA) Control Region Through Massive Parallel Sequencing (MPS) Analysis**

*Sidney Gaston Sanchez\**, Pennsylvania State University, State College, PA 16802; *Charity A. Holland, MPH*, Pennsylvania State University, University Park, PA 16802; *Mitchell M. Holland, PhD*, Pennsylvania State University, University Park, PA 16802

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**Learning Overview:** After attending this presentation, attendees will gain insight on the effects of hydrolytic DNA damage observed on mitochondrial DNA (mtDNA) sequenced through Massive Parallel Sequencing (MPS) and the potential value in repairing damaged extracts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving analysts' ability to identify DNA damage. If extracts are designated as damaged, applying a repair cocktail prior to library preparation and sequencing can reduce damage as an artifact downstream in the interpretation process.

After attending this presentation, attendees will gain insight on the effects of hydrolytic DNA damage observed on mitochondrial DNA (mtDNA) sequenced through massive parallel sequencing (MPS) and the potential value in repairing damaged extracts.

DNA damage can impact the interpretation of sequence-based mtDNA profiles, including when interpreting heteroplasmy. Hydrolytic DNA damage can be observed in forensic and ancient DNA samples and can result in lesions and both single- and double-stranded breaks. Two common products of hydrolytic DNA damage are deamination and depurination. Deamination can occur through hydrolysis of functional groups on nitrogenous bases resulting in a base change, while depurination occurs through cleavage of the glycosidic bond between the base and the sugar moiety, creating an abasic site.

In this study, DNA extracts of donors with known haplotypes were hydrolytically damaged by incubating them in water at varying time periods and temperatures. The extracts were then repaired with the NEBNext® Formalin-Fixed Paraffin-Embedded (FFPE) DNA Repair Mix and the mitochondrial control region was sequenced through MPS analysis. Samples incubated at 37°C resulted in extensive degradation, a product of severe damage, while those incubated at room temperature exhibited random deamination-like and depurination-like events. Degradation increased in samples that were incubated for longer time periods. In every experiment, non-repaired portions of extracts had more damage sites than repaired portions of extracts; the rate of damage sites per 100 nucleotides (nt) was higher in the non-repaired samples when compared to the repaired samples (0.31-0.52 vs 0.01-0.29 damage sites/100 nt, respectively). While the repair cocktail decreased the amount of existing damage sites, it did not completely eliminate them. Therefore, damage variants should be expected when working with forensic evidence that may complicate the interpretation of heteroplasmy. To mitigate this effect, replicate amplifications are recommended, as this resulted in no duplicate sites of damage in the current study, consistent with numerous previous studies.

Future experiments will focus on a single incubation time and temperature to assess the reproducibility of damage patterns and the performance of the repair method. Based on our collective findings, we will assess the overall value of conducting the additional step of repairing DNA extracts before MPS mtDNA sequencing from a modeling perspective.

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#### **Hydrolytic DNA Damage, Repair, Mitochondrial DNA**



### **B107 Massively Parallel Sequencing (MPS) and Short Tandem Repeat (STR) Analysis of Human DNA From Partial Bloody Fingerprints Enhanced With Columnar Thin Films (CTF)**

*Teresa M Tiedge\*, Pennsylvania State University Park, PA ; Nivedita Nagachar, PhD, Forensic Science Program, University Park, PA 16802; Akhlesh Lakhtakia, PhD, DSc, University Park, PA 16802; Reena Roy, PhD, Pennsylvania State University, University Park, PA 16802*

**Learning Overview:** After attending this presentation, attendees will understand how CTF development is conducted using the Conformal-Evaporated-Film-By-Rotation (CEFR) method on partial bloody fingerprints. STR analysis completed on these types of samples will demonstrate to the audience, that certain evaporant materials do not inhibit Polymerase Chain Reaction (PCR) or capillary electrophoresis. Additionally, presentation of the data from Single Nucleotide Polymorphism (SNP) genotypes will inform the audience on new technologies that can be implemented in crime laboratories.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by focusing on new DNA analysis methods and the non-traditional enhancement of fingerprints.

Fingerprints are commonplace on various substrates at crime scenes. Traditional methods of enhancing latent fingerprints include cyanoacrylate fuming and dusting with carbon-based, fluorescent, magnetic, or other powders. Enhancement of partial bloody fingerprints is challenging because the latent and the patent components require different methods that may be difficult to cascade. Deposition of a columnar thin film (CTF) on partial bloody fingerprints has been shown to be effective for some types of forensically relevant substrates. CTF deposition requires the use of the conformal-evaporated-film-by-rotation (CEFR) method, allowing for conformal growth of CTFs from the fingerprint. Prior research with deposition of CTFs of Alq<sub>3</sub> on partial bloody fingerprints on brass has established that CTF deposition preserves DNA for short tandem repeat (STR) DNA analysis.

Recent advances in massively parallel sequencing (MPS) have made sequencing more economical and faster compared to earlier technologies. Single nucleotide polymorphisms (SNPs) are advantageous for use with low-quality samples because their amplicon size is smaller than that of STRs. MPS technology, in combination with SNPs, can be helpful in identifying DNA profiles from low-quality samples such as fingerprints. The primary goal of this research is to combine fingerprint enhancement with CTFs, and DNA analysis with MPS allowing for dual identification of an individual, thereby strengthening evidentiary value. Additionally, MPS libraries may be prepared manually or through automation. A secondary goal of this research was to compare sequencing data between the two library preparation methods.

Partial bloody fingerprints collected on glass, brass, cherry wood, black garbage bags, and clear sandwich bags were used in this project. CTFs of Alq<sub>3</sub>, gold, Eu(tta)<sub>3</sub>phen, or GeSbSe chalcogenide glass, as appropriate, were deposited on the samples. DNA was extracted from undeveloped as well as CTF-developed fingerprints. Quantification using qPCR was performed to determine the degradation index of every sample. In addition to STR testing, DNA extracts were also sequenced on the Ion S5™ to determine SNP genotypes. The Precision ID Identity Panel contains primers for 124 SNPs and consists of 90 autosomal and 34 Y-clade SNPs. The Ion Chef™ was used to prepare the libraries via automation, as well as to template the libraries onto the semi-conducting chip for sequencing. This study demonstrated that CTF nanotechnology can be used to individualize humans using both STR and MPS techniques. It was determined that the use of gold, chalcogenide glass, and Eu(tta)<sub>3</sub>phen as evaporant materials were not inhibitory to STR analysis. It was concluded that automated and manual library preparations have different advantages, and laboratory throughput may influence which method is implemented.

*This work was partially supported by Grant No. 2016-DN-BX-0153 from the U.S. Department of Justice.*

### **Massively Parallel Sequencing, Columnar Thin Films, Partial Bloody Fingerprints**

## **B108 A Newly Developed Massively Parallel Sequencing (MPS) Microhaplotype Forensic Assay for Mixture Detection and Deconvolution and Ancestry Prediction**

Fabio Oldoni, PhD\*, The George Washington University, Washington, DC 20007; Drew A. Bader, AB, Washington, DC 20007; Sharon C. Wootton, PhD, South San Francisco, CA 94080; Robert Lagacé, BS, Thermo Fisher Scientific, South San Francisco, CA 94080; Ryo Hasegawa, BS, Foster City, CA 94404; Joseph P. Chang, BS, Thermo Fisher Scientific, South San Francisco, CA 94080; Kenneth Kidd, PhD, Yale University School of Medicine, New Haven, CT 06520; Daniele S. Podini, PhD, Department of Forensic Science, Washington, DC 20007

**Learning Overview:** After attending this presentation, attendees will understand the use of massively parallel sequencing (MPS) for the analysis of microhaplotypes (MHs).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting a new forensically relevant MPS-MH typing approach to enhance both mixture deconvolution and ancestry prediction.

Microhaplotypes are markers defined by two or more SNPs located within a short distance from each other (<300 bp) associated in multiple allelic combinations within a locus. These markers have the potential to improve human identification, enhance mixture deconvolution capabilities while enabling for ancestry inference.<sup>1-3</sup> In addition, MHs have some advantages over short tandem repeats (STRs) including the absence of stutter peaks, alleles within a locus all having the same size, and a lower mutation rate than conventional forensic markers. Altogether they can achieve discrimination power like that of STRs while providing a greater amount of information. Sanger sequencing is unable to determine the *cis/trans* relationship of SNP alleles within a MH locus while MPS enables determining the parental haplotypes at each locus by clonal sequencing of each DNA strand.<sup>3</sup> In this study, we defined the detection limit of a novel panel of 74 MH loci analyzed on the Ion Chef™/Ion S5™ (Thermo Fisher Scientific) MPS platform, explored the assay efficiency in mixture analysis and compared the results to conventional capillary electrophoresis (CE)-STR typing, and evaluated ancestry inference capabilities of the detected minor contributor.<sup>4</sup>

The sensitivity limit of the MPS-MH assay was tested by typing three samples in triplicate from 2 ng to 25 pg input DNA range. The sensitivity and mixture studies were conducted in parallel comparing MPS of MHs and CE of STRs (GlobalFiler™ kit, Thermo Fisher Scientific) on the same mixed samples. The analysis of MH and STR mixtures was performed using a wide range of artificial mixed-source samples to mimic forensic scenarios commonly encountered in caseworks. These included approximately 100 simulated two-to-five-person mixtures at 1-10 ng input DNA, with each donor having a distinct ancestry origin and contribution ratio. Representative examples of tested ratios included 10:1 to 80:1 for two-person mixtures, 10:1:1 and 5:5:1 for three-person, 10:1:1:1 and 5:5:1:1 for four-person mixtures, and 10:1:1:1:1 and 5:5:1:1:1 for five-person mixtures. For genotyping of MH loci, the latest released Microhaplotyper Plugin v8.1 (Thermo Fisher Scientific) was used. In addition, a set of approximately 400 individual representatives of four major American population groups (US African, US European, US Asian, and South West Hispanics) was genotyped and allele frequency tables for estimating the biogeographic ancestry of the minor donor were generated.

Overall the MPS-MH assay was sensitive down to 50 pg input DNA with minimal allele dropout at 25 pg input DNA. For two-person mixtures, full MH profile of the minor donor was reported at a 1:10 ratio while few allele dropouts were observed at a 1:20 ratio. Moreover, the random match probability (RMP) calculated for the minor donor was higher than that obtained for the same mixtures analyzed with STRs. For the three-to-five-person mixtures, full MH profile was reported for all minor donors within the full range of mixture ratios tested. For these mixtures, STR profiles of the minor donors were fully or partially detectable. However, due to the level of complexity of the mixtures the minor contributor(s) would have been considered not suitable for comparison. In addition, ancestry of the minor donor of two-person mixtures was correctly predicted by dividing the highest RMP value obtained using different populations by the second and third highest. The value obtained is indicative of how much more likely it is to observe the profile of interest if it originated from an individual from the population at the numerator than if it originated from an individual from the population at the denominator.

These results suggest that the 74plex MPS-MH assay is an effective and versatile forensic tool, which allows for mixture deconvolution and ancestry inference. Current work is also focused on the comparison of MPS-MH to MPS-STR typing on the same mixed samples.

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1. Kidd KK, Speed WC. Criteria for selecting microhaplotypes: mixture detection and deconvolution. *Investigative genetics* (2015) 6(1):1.
2. Kidd KK, Pakstis AJ, Speed WC, Lagacé R, Chang J, Wootton S, Haigh E, Kidd JR. Current sequencing technology makes microhaplotypes a powerful new type of genetic marker for forensics. *Forensic Science International: Genetics* (2014) 12:215-224.
3. Kidd KK, Speed WC, Pakstis AJ, Podini DS, Lagacé R, Chang J, Wootton S, Haigh E, Soundararajan U. Evaluating 130 microhaplotypes across a global set of 83 populations. *Forensic Science International: Genetics* (2017) 29:29-37.
4. Oldoni F, Hart R, Long K, Maddela K, Cisana S, Schanfield M, Wootton S, Chang J, Lagace R, Hasegawa R, Kidd K, Podini D, Microhaplotypes for ancestry prediction (2017) *Forensic Science International Genetics Supplement Series* 6: e513-e515

### **MPS of Microhaplotypes, Mixture Deconvolution, Ancestry Inference**





### **B109 FONTANA: A FOrensic NexT-Generation ANALysis Pipeline for High-Throughput Microhaplotype Data Analysis**

*Keylie M. Gibson, BS\*, The George Washington University, Ashburn, VA 20147; Fabio Oldoni, PhD, The George Washington University, Washington, DC 20007; Rebecca M. Hart, The George Washington University, Washington, DC 20007; Daniele S. Podini, PhD, Department of Forensic Science, Washington, DC 20007; Keith A. Crandall, PhD, The George Washington University, Washington, DC 20052*

**Learning Overview:** After attending this presentation, attendees will become familiar with a new computational software tool specifically designed for their community—FONTANA: the FOrensic NexT-generation ANALysis pipeline. Attendees will discover the strengths of this new approach to analyze next-generation sequencing data for microhaplotype discovery and analysis and experience applications of this new approach to improve human identification efforts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing the community to a new computational tool for analyzing next-generation sequencing data focused on microhaplotypes. Additionally, this presentation will help bridge the gap between forensic science and computational biology.

Microhaplotypes (MHs), typed on Next-Generation Sequencing (NGS) platforms, can enhance mixture deconvolutions and provide increased discrimination power and ancestry predictions. MHs are loci of two or more single nucleotide polymorphisms (SNPs) within a short distance from each other (<300 nucleotides i.e. ‘micro’) with three or more allelic combinations (‘haplotypes’). Unlike STRs, MHs have a low mutation rate and show no PCR stutter, and using NGS, phasing information between the SNPs can be attained. The amount of data generated by a single NGS run can be upwards of a million times greater (4 billion base pairs to 4 trillion base pairs or 1GB to 1TB of data) compared to about 4 million bases pairs (or 1MB of data) generated by a single Sanger sequencing run. Working with data on this scale requires new computational tools. A tool has been developed to assist the forensic science community with MH analyses. Introducing FONTANA: FOrensic NexT-generation ANALysis pipeline. FONTANA is a working pipeline, platform agnostic, created to analyze microhaplotypes from forensic samples. FONTANA currently consists of quality control, alignment to the human genome reference, variant calling, haplotype calling, and report generation. Quality control is executed by Flexbar, where low quality reads, low quality nucleotides, and adapter sequence contamination are removed. The sequencing reads are then aligned to the human genome reference, and ready for the next step: variant calling, completed with FreeBayes. Haplotypes, characterized by phasing the SNPs together, are called with the FreeBayes program for each sample from the previous step where all variants (at known and unknown SNP location) were identified. This entire pipeline is executed in the Snakemake workflow, which is a tool to create reproducible and scalable data analyses—ideal for a forensic application. FONTANA’s environment has been configured in Bioconda. Bioconda is a package manager for bioinformatic software, and therefore, FONTANA can be accessible to a variety of operating systems (Linux and Mac OSX). An initial version of FONTANA was applied to three populations: Mexican Pima, European American, and Southwest Hispanic, each with 50 individuals, with ten microhaplotypes. The most unique alleles found at a single MH was 14, with a range between two to 14 alleles at a MH (median = 5 alleles). One of the microhaplotypes showed that admixed populations (Southwest Hispanic and European American) contain a greater diversity of alleles present than in a homogeneous population (Mexican Pima). Moreover, utilizing MHs facilitates biogeographic ancestry prediction in a sample. Over 70 MHs will be added to the pipeline; additionally, the goal of FONTANA is to be designed as a plastic pipeline, so that as new and more informative MHs are discovered, they can easily be added to FONTANA. FONTANA can be found at <https://github.com/kmgibson/FONTANA>, a GitHub page developed for tracking the progress and usage and providing guidelines for execution of the software program. Future directions include adding downstream applications for ancestry prediction, mixture deconvolution, and probabilistic genotyping. The development of this computational analysis tool and identifying additional SNPs within each MH strengthens the foundation of MHs for use in criminal casework and will help combat the problem of mixture deconvolution.

#### **Microhaplotypes, Computational Software, Next Generation Sequencing**



### **B110 The Use of Receiver Operating Characteristic (ROC) Curves as a Tool to Assess Noise and Zygosity in the Targeted Sequencing of Forensic Short Tandem Repeat (STR) Markers**

*Sarah Riman, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD 20899; Hariharan Iyer, PhD, Gaithersburg, MD; Lisa Borsuk, MS, National Institute of Standards and Technology, Gaithersburg, MD 20899; Peter M. Vallone, PhD, National Institute of Standards and Technology, Gaithersburg, MD 20899-8314*

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**Learning Overview:** After attending this presentation, attendees will understand how to characterize and understand noise, stutter artifacts, heterozygote imbalance, allelic drop-in, and allelic drop-out in Next Generation Sequencing (NGS) datasets generated from single-source samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing a framework of statistical tools developed to systematically interpret and understand the characteristics of single-source DNA profiles generated by targeted sequencing.

The sequencing of STR markers provides additional information due to the underlying sequence variation that is typically masked by traditional fragment-based genotyping. The interpretation of STR profiles generated by targeted sequencing methods are susceptible to familiar parameters such as signal noise, stutter artifacts, heterozygote imbalance, and allelic drop-out/in, as well as additional factors introduced by the library preparation workflow.

In this work, data were generated from sensitivity studies using known single-source samples. The DNA extracts were amplified with the PowerSeq 46GY System Prototype with varying DNA target masses ranging from 15 pg to 500 pg. Amplified PCR products were subjected to library preparation using two different library preparation kits: Truseq DNA PCR-Free High Throughput (HT) Library Prep Kit (Illumina) and KAPA Hyper Prep Kit (KAPA Biosystems). Libraries were either normalized or left without normalization. Paired-end sequencing of the STR loci was then performed on the Illumina MiSeq platform, and raw FASTQ data files were analyzed using a modified version of the open source STRait Razor v2.0. The software identified the sequences, allele length, and coverage of the STR markers and regions at a minimum depth of coverage of 1X to capture as much data as possible. Receiver Operating Characteristic (ROC) curves were then used to understand the tradeoff between true positives (alleles) and false positives. False positives were attributed to drop-ins, stutter, and random noise. ROCs were also used to infer and examine zygosity using heterozygote balance (Hb) information to minimize the risks of misidentifying a heterozygote as a homozygote locus or a homozygote as heterozygote locus. Data generated from each library workflow were analyzed globally (all DNA quantities combined), as well as investigated per DNA quantity and per locus.

The aim of this presentation is to share the findings and show how analyses presented here can also be applied to sequence data generated by similar targeted sequence multiplexes and/or sequencing platforms.

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#### **Receiver Operating Characteristic, Noise, Next Generation Sequencing**



### **B111 Eye, Hair, and Skin Color Prediction Using the HIrisPlex-S (HPS) System and Massively Parallel Sequencing (MPS)**

*Krystal Breslin, MS, Fairfax, VA 22032-2438; Bailey M. Wills, BS\*, Indianapolis, IN 46224; Susan Walsh, PhD, Indiana University Purdue University Indianapolis, Indianapolis, IN 46202*

**Learning Overview:** After attending this presentation, attendees will understand the field of Next Generation Sequencing (NGS) technologies by exploring the capabilities of the integration of the HIrisPlex-S (HPS) System and Massive Parallel Sequencing (MPS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by outlining the advantages of Next Generation Sequencing technologies and illustrate how they can be used successfully in DNA phenotyping to help as investigative leads in forensic casework, anthropological cases, and missing persons cases.

Forensic DNA Phenotyping acts as a “biological eyewitness.” It can predict externally visible characteristics from a small sample of DNA, and provide investigative leads in anthropological cases, missing person cases, and forensic casework. Fundamental genetics research has led to a better understanding of the specific variants responsible for physical appearance characteristics, particularly eye, hair, and skin color. With this knowledge, the prediction model system termed ‘HIrisPlex-S’ with model parameters generated from thousands of individuals combines the categorical prediction of these three pigmentation traits in one using only 41 variants of DNA. Currently, average prediction accuracies are approximately 95% for blue or brown eye color, 83% for red, blond, brown, or black hair color and 86% for very-pale, pale, intermediate, dark or dark-to-black skin color. The most probable pigmentation profile can be obtained once these variants are uploaded to the freely accessible online prediction tool found at <https://hirisplex.erasmusmc.nl>.

At present, these 41 variants can be generated from two developmentally validated capillary electrophoresis assays to generate the required input genotypes.<sup>1,2</sup> However, due to next generation sequencing technologies and the use of Massive Parallel Sequencing (MPS) in assay design, here the authors describe a validated MPS assay for use on both the Ion Torrent and MiSeq systems that is capable of producing fragments that incorporate all HIrisPlex-S variants in one sensitive assay. By integrating the HIrisPlex-S system with a fully automated sequencing pipeline, high throughput data can be generated and uploaded to the on-line tool for an easier workflow at a lower cost. In addition, due to the nature of sequencing and the generation of data surrounding the required prediction variant, it allows the capacity to better deconvolute mixtures. It is also one step closer to adding all known physical appearance prediction markers, beyond pigmentation, into a single library preparation for Massive Parallel Sequencing, creating a true biological sketch. The HPS-MPS assay now overcomes a large hurdle in forensic DNA phenotyping as physical appearance predictions can now be possible with mixtures in addition to single source DNA profiles.

#### **Reference(s):**

1. Walsh et al., *Developmental validation of the HIrisPlex system: DNA-based eye and hair color prediction for forensic and anthropological usage*. Forensic Science International Genetics 9: 2013.
2. Chaitanya et al., *The HIrisPlex-S system for eye, hair and skin color prediction from DNA: Introduction and forensic developmental validation*. Forensic Science International Genetics 35: 2018.

#### **Phenotype, Prediction, Sequencing**



### **B112 The Examination of DNA Extraction Lysis and Wash Step Modification for Low Template DNA Sample Processing**

*Sydney Menchhoff\**, Virginia Commonwealth University, McLean, VA 22101; *Milady T. Delacruz*, Virginia Commonwealth University, Richmond, VA 23284; *Madison Hytinen, BS*, Virginia Commonwealth University, Richmond, VA 23220; *Marilyn T. Miller, EdD*, VA Commonwealth University, Richmond, VA 23284-3079; *Tracey Dawson Cruz, PhD*, Virginia Commonwealth University, Richmond, VA 23284

**Learning Overview:** After attending this presentation, attendees will have a better understanding of alternative methods of DNA extraction that can be used to potentially increase overall DNA yields and improve short tandem repeat (STR) profiles of DNA extracted from archived latent fingerprints.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring methods that can be used in forensic laboratories to increase the success rate of obtaining a profile from low copy number fingerprint samples.

In recent years, as DNA analysis methods have become more sensitive, more attention has been paid to reevaluating closed and/or cold cases. In some of these cases, archived latent fingerprints may be the only source of biological evidence to revisit. Previous work on this project explored a variety of sampling techniques, extraction kits, and purification methods to establish an optimized “best practice” workflow for these challenging samples. Those studies found that directly extracting cuttings of the tape *and* paper sides of an archived print using QIAGEN QIAamp® DNA Investigator Kit, followed by quantification, pre-amplification purification via Centri-Sep™ columns, and concentration via vacuum centrifugation produced the best DNA yields and STR profiles. However, that study was completed using recently collected archived latent fingerprints; it is suspected that older samples may be more challenging. Thus, it is important to examine other potential procedural modifications that may minimize the DNA loss that occurs during laboratory handling of these samples. A preliminary study was completed to more closely examine where DNA loss occurs during the extraction process. On average, 83 ng of DNA (4.3% of the total DNA from each original buccal swab processed) did not bind to the column and was eluted with the initial lysate flow-through. This was enough DNA to obtain full STR profiles for 9 of 10 lysate flow-through samples tested. Consequently, a study was completed using 20 aged (2 years) archived latent fingerprint samples that were extracted using the QIAGEN QIAamp® DNA Investigator Kit, but the initial lysate filtrate was kept and extracted separately. The DNA extract and lysate filtrate samples from each individual sample tested were combined after quantification; combined samples were again quantified then concentrated and amplified (as a single sample) following the optimized methods previously described. While this modification increased DNA yields by 30% (from 0.183 ng to 0.263 ng), combining these samples reduced the number of expected STR alleles by 27% when compared to aged samples that were processed without the modification. Subsequently, a second study was conducted using a “double lysis” technique, with the aim of maximizing cell lysis and exposing more DNA for purification. For this technique, another 20-aged archived latent fingerprints (magnetic-powder treated and untreated) were processed using the same extraction method, but with one modification – after the initial lysis step, both the tape and paper sides of the sample were lysed twice and both lysates were combined for DNA extraction. Although this modification led to more samples producing detectable DNA, a more than 2-fold decrease in total DNA yields and a 22% decrease in the number of detected STR alleles were observed overall when compared to aged samples that were processed without the modification. Interestingly, though not improved with the method modification, magnetic-treated samples specifically were not affected, producing approximately the same number of expected STR alleles regardless of the lysis procedure used. Overall, our results suggest that while some DNA is lost during the laboratory extraction process, the amount recoverable may not be sufficient to improve the STR profile obtained from archived latent fingerprint samples using DNA extraction procedure modifications.

#### **DNA Loss, Low Template DNA, Fingerprints**



### B113 Standardizing Testing for DNA Shedding Propensity

Mechthild K. Prinz, PhD\*, John Jay College of Criminal Justice, New York, NY 10019; Marine Bougerie Le Du, Osny, Val D'oise, FRANCE; Dinura Gunatilake, BS, John Jay College, New York, NY 10019; Nicholas D. Petraco, PhD, John Jay College of Criminal Justice, New York, NY 10019-1007

**Learning Overview:** After attending this presentation attendees will: (1) be aware of the impact of an individual's shedding propensity on their DNA transfer risk, and (2) have learned about a proposed method to test this for research and casework subjects.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a standardized test for shedding propensity that can provide crucial information for the assessment of secondary transfer risks. Implementing this type of testing in casework could support expert testimony in this area.

With the majority of DNA casework now consisting of contact DNA traces, the distinction between active versus passive DNA transfer is important for the probative value of this type of evidence. A recent paper on transfer via handshakes concluded that it was not different preceding activities but "the relative shedding ability" of the two volunteers that had the largest effect on whose DNA was detected.<sup>1</sup> Instances where passive transfer generates a single source profile but not from the person touching, can generally be explained by the detected DNA being from a high shedder.<sup>2</sup> This means, knowing the shedder status of a person of interest would greatly benefit evidence interpretation in a specific case. Published methods on shedder testing show a wide variability of parameters regarding touched substrate, duration and area of contact, time after handwashing, recovery techniques, and DNA extraction. The method tested here avoids deposit and recovery variation by using tape discs with a defined collection area and employs a widely available DNA extraction kit.

Samples were collected from male and female volunteers using hypoallergenic D-Squame adhesive tape disks (D100, CuDerm Corporation, Dallas, TX), and by having them touch a sterile 50mL polypropylene tube (Corning Falcon tubes, Corning, NY) for 15 seconds. Several sets of parallel samples from the same donor were collected for touched Falcon tubes and the following skin areas: fingers and thumbs 15 minutes after hand washing, thumbs without washing, lower inside arm, and back shoulder. The sebaceous skin areas were collected to test for a possible correlation to palmar skin. DNA was extracted using QIAmp DNA Investigator kits (Qiagen, Germantown, MD) and quantitated with Quantifiler Trio (Thermo Fisher Scientific Applied Biosystems, Carlsbad, CA).

As in previous studies, samples from male individuals contained on average more DNA than samples from females but also showed a larger standard deviation.<sup>3</sup> With the exception of shoulder samples, this difference was not statistically significant. Tape lifts from unwashed thumbs contained the most DNA, followed by fingers after handwashing without soap, and the shoulder area. Touched Falcon tubes yielded the least amount of DNA. Correlation testing between parallel samples showed a strong correlation between finger and thumb lifts from the same hand after washing. This demonstrates the reproducibility of the collection method. There was also a correlation between washed fingers and amount of DNA deposited on Falcon tubes, meaning tape lift results are representative of what would be left behind when touching an object. More samples will be needed to strengthen these conclusions. Another future research topic is the question, if a single collection event can truly detect an individual's biological shedder propensity, or if this is only a transient quality always dependent on daily activities and other circumstances.

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1. Szkuta B, Ballantyne KN, Van Oorschot RAH. Transfer and persistence of DNA on the hands and the influence of activities performed. *Forensic Sci Int Genet.* 2017;28:10–20.
2. Lowe A, Murray C, Whitaker J, Tully G, Gill P. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *Forensic Sci Int.* 2002;129:25–34.
3. Lacerenza D, Aneli S, Omedei M, Gino S, Pasino S, Berchiolla P, et al. A molecular exploration of human DNA/RNA co-extracted from the palmar surface of the hands and fingers. *Forensic Sci Int Genet.* 2016;22:44–53.

#### DNA Transfer, DNA Shedder, Tape Lifts



### B114 Improved DNA Recovery From Handwritten Documents

Patrick McLaughlin, BS\*, John Jay College of Criminal Justice, New York, NY; Mechthild K. Prinz, PhD, John Jay College of Criminal Justice, New York, NY 10019

**Learning Overview:** After attending this presentation, attendees will be aware of a new non-destructive method of vacuum swabbing large surface areas of handwritten notes, and how it expands DNA testing for paper evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into a technique for processing regions of paper evidence typically overlooked in current DNA testing strategies. The promising DNA success rates and its non-destructive nature make this method a valuable addition to the forensic science toolbox.

When paper evidence is submitted for evidence recovery, a common practice is to prioritize the chemical or physical development of latent prints prior to attempting any of the various methods of DNA extraction from the prints. Areas of the periphery are commonly targeted for print visualization where people most commonly hold and lift sheets of paper. The written text is rarely considered an area of interest for criminalists but has the potential to retain “touch” DNA evidence deposited when the writer of scraped their hand across the page during the writing process. Several published papers have indicated that there are varying levels of DNA capable of being recovered from different parts of the hand. However, the protracted contact between skin and the non-porous paper surface may provide enough biological evidence to generate a complete STR profile. The method tested here was designed to collect potential DNA from the surface of a handwritten note from inside the text area as opposed to the edges.

Samples were collected from male and female volunteers who were asked to write a copy of a provided note, as well as provide a reference buccal swab. Additionally, a “field case” study was performed where volunteers provided a writing sample prior to and after a mild aerobic exercise period. The purpose of this test was to simulate an apparent excited state which a criminal might be experiencing while writing threatening notes or bank robberies. The writing samples were then vacuumed using an irradiated and trimmed Carolina 9-inch glass pipette containing a moistened cotton Puritan swab. With a vacuum hose attached to the narrow end of the pipette, the entire text area of the paper was systematically processed. DNA was extracted using 5% Chelex, 10% Tween-20, and Proteinase K and concentrated using Microcon membrane filter units.<sup>1</sup> All recovered samples were quantified using Quantifiler Trio and typed with AmpflSTR Identifiler Plus (both Thermo Fisher Scientific Applied Biosystems, Carlsbad, CA).

The quantity of recovered DNA varied greatly from donor to donor, but most of the collected samples provided sufficient material to produce profiles which could be successfully compared to the reference samples. This technique enhances the ability to recover DNA evidence which may have been otherwise destroyed or damaged during the latent print visualization process. Additionally, the process does not damage the document, which may be of importance for prosecutorial value and court demonstration.

#### Reference(s):

<sup>1</sup>. Forsberg C, Jansson L, Ansell R, Hedman J. High-throughput DNA extraction of forensic adhesive tapes. *Forensic Sci Int Genet.* 2016;24:158–63.

#### Paper, Contact DNA, Recovery



### B115 Improving Recovery of Trace DNA From Cotton Swabs Using Pressure Cycling and Alkali-Based Lysis

Meghan N. Roig, MSFS\*, Florida International University, Miami, FL 33194; Nicole Fernandez Tejero, Ponce, PR 00732; Vanessa Martinez, Miami, FL 33125; Bruce R. McCord, PhD, Florida International University, Miami, FL 33199

**Learning Overview:** After attending this presentation, attendees be introduced to a new application of an efficient method for lysing and extraction low amounts of epithelial cells.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing results for a method that can decrease the analysis time for trace amounts of epithelial cells. Attendees will see results of a pressure and alkali-based protocol originally designed for differential lysis optimized for the recovery of trace samples. These results provide a method to efficiently lyse and recover trace amounts epithelial cells and DNA.

Due to recent improvements in sensitivity of DNA test procedures, more and more samples consist of low level, single-source DNA and mixtures. Low levels of input DNA can result in problems with amplification, such as heterozygous peak imbalance, allelic drop out, and drop in. Much of a sample can be lost during extraction, leading to even less sample available for the amplification process.<sup>1</sup> DNA extraction from cotton swabs have been found to result in a 20-76% drop in recovery.<sup>2</sup> This loss occurs at various stages in the extraction, most commonly during wash and transfer steps. However, another major contributing factor is the irreversible adsorption of cells to the cotton matrix. This study demonstrates improved recovery of DNA using pressure cycling and alkaline lysis. This process recovers more DNA through chemical modification of cotton and pressure assisted disruption of cells.

In this study, a method for differential lysis of epithelial and sperm mixtures has been modified to improve the recovery of trace samples. Increasing temperature and modifying the amount of NaOH lead to an improved recovery of trace amounts of DNA. Cotton swabs were treated with 800 $\mu$ L 0.05N NaOH at 55°C for 10 cycles of 15 seconds at 20kpsi and 15 seconds at ambient pressure. This removed and lysed epithelial cells from the cotton swab in 5 minutes. The lysates are neutralized and concentrated with a DNA filter. The samples were quantified using *Alu*-based real-time PCR.

Results demonstrate high recovery of DNA from a small number of cells. Over 60% of DNA was recovered from samples containing 1,500 to 50 cells, with about 50% of DNA recovered from samples containing 20 cells. These methods were developed based off an experimental design software that used multivariate response methods to optimize the parameters of the extraction for improved recovery of DNA. Additional optimization of the technique was guided by a multivariate experimental design to discern which parameters would elicit the largest increase in DNA recovery.

#### Reference(s):

1. Adamowicz MS, Stasulli DM, Sobestanovich EM, Bille TW. Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. *PloS one*. 2014;9(12):e116351.
2. Van Oorschot R, Ballantyne KN, Mitchell RJ. Forensic trace DNA: A review. *Investig genet*. 2010;1(1):14.

#### Pressure Cycling Technology, Trace DNA, DNA Recovery



### **B116 The Assessment of Variable Elution Volumes for Efficient Recovery of Low-Level DNA Samples From Robotic Extraction Platforms**

*Heather V. Milnthorp, MSFS\*, Center for Forensic Science Research & Education, Willow Grove, PA 19090; Yih Ling Saw, MSFS, Center for Forensic Science Research & Education, Willow Grove, PA 19090; Heather E. McKiernan, MSFS, Center for Forensic Science Research & Education, Willow Grove, PA 19090*

**Learning Overview:** After attending this presentation, attendees will better understand the complexities and challenges associated with the extraction, amplification, and analysis of low-level sample types such as touch DNA. Attendees will also learn techniques for manipulating the built-in feature of variable elution volumes with commonly employed robotic DNA extraction platforms to increase the quantity of DNA which can be recovered from low-level samples, subsequently increasing the probability of generating robust, highly-discriminatory Short Tandem Repeat (STR) profiles.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an efficient modern workflow solution for maximizing the amount of discriminating genetic information which can be produced from challenging low-level sample types such as touch DNA samples.

As the sensitivity of downstream processes in the DNA workflow continues to increase, case-working labs are seeing an increase in the number of samples being submitted for analysis with a concurrent decrease in the quality of samples being submitted. Many submissions are the result of property crimes or cold-cases, where there may be minimal traces of DNA amount of available sample remaining for retesting. To that end, it is imperative that front-end processes such as extraction can perform with increased stringency and efficiency. Recently, some of the major manufacturers of robotic DNA extraction platforms have released updated script cards allowing the end-user greater flexibility for elution volume used when extracting DNA samples. These options may prove beneficial in increasing the amount of genetic information which can be recovered from low-level challenging sample types, such as touch DNA.

The goal of the current research was to demonstrate a linear relationship between the elution volume applied on a robotic DNA extraction platform and total DNA yield in a sample. Using the Applied Biosystems® PrepFiler Express™ Forensic DNA Extraction Kit, a 1% serial blood dilution was extracted in triplicate on the Applied Biosystems® AutoMate Express™ DNA Extraction System. Following extraction, all samples were quantified using the Life Technologies Quantifiler® Trio quantification kit on an Applied Biosystems® 7500 Real-Time PCR System. Analysis of the quantitative data produced, as expected, a strongly negative correlation between increasing elution volume and decreasing sample concentration ( $r = -0.815$ ,  $p = 0.0254$ ). Calculation of total DNA yield resulted in a strongly positive correlation between increasing elution volume and increasing total DNA yield of the sample ( $r = 0.798$ ,  $p = 0.0316$ ). Average total DNA yield of the sample eluted at 20  $\mu\text{L}$  (5.555 ng) was significantly lower than the average total DNA yield of the sample eluted at 250  $\mu\text{L}$  (7.302 ng). A single-factor analysis of variance revealed a statistically significant difference in total DNA yield recovered at variable elution volumes ( $F_s = 4.517$ ;  $df = 6, 20$ ;  $p = 0.0094$ ).

This study demonstrates that by increasing the elution volume applied to suspected low-level DNA samples, more total DNA can be recovered. Previously completed work has demonstrated the reliability of an in-house pre-amplification concentration protocol, utilizing an Eppendorf Vacufuge® plus vacuum concentrator. This data suggests that eluting low level DNA samples in higher elution volumes followed by pre-amplification concentration may allow for increased likelihoods of obtaining more complete, robust STR profiles as compared to eluting in lower volumes. Further work is currently being conducted to rigorously assess the quantitative and qualitative performance of low-level samples utilizing the proposed workflow with variations in buffer solution and robotic extraction platform selection.

#### **Robotic DNA Extraction, Elution Volume, Quantitative Recovery**





### B117 A Proteomic Analysis of Epidermal Squamous Corneocytes

*Trevor A. Borja, BA\*, San Jose, CA 95118; Noreen Karim, PhD, University of California, Davis, Davis, CA 95616; Zachary C. Goecker, MPS, Visalia, CA; Michelle Salemi, MS, UC Davis Proteomics Core, Davis, CA 95616; Brett Phinney, PhD, UC Davis Proteomics Core, Davis, CA 95616; Robert Rice, PhD, University of California, Davis, Davis, CA 95616; Glendon Parker, PhD, University of California, Davis, Davis, CA 95616*

**Learning Overview:** After attending this presentation, attendees will learn about proteomics and how it can be used as a potential means of human identification. Attendees will also learn how bottom up discovery allows for inference of genotypes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a potential new tool for consideration when DNA alone is insufficient, as it often is in touch evidence and fingerprints.

The DNA found in biological samples collected as evidence is not always a sufficient source of genetic information. An additional application of proteomics can allow for the expansion of identifying information available to the forensic investigators. Proteins can be an even better source at times because of its greater stability and abundance. Protein-based human identification experimentation begun with studies on peptides generated from hair. Recent advancements in instrumentation and bioinformatic tools have added further to this approach. The experimental focus is on the detection of genetically-variant peptides (GVPs) that result from changes in an amino acid sequence. Further, these single amino acid modifications are seen when variation exists in protein encoding genes as non-synonymous single nucleotide polymorphisms (nsSNPs). Thus, identification of single amino acid polymorphisms (SAPs) allows for DNA content to be inferred.

To demonstrate the utility of proteomics in forensic science, the protein population of skin cells was thoroughly examined and compared with respective exome data. This is significant because DNA found in touch evidence and fingerprints is often fragmented or degraded. A method has been developed to detect peptides of epidermal squamous corneocytes (ESCs) to catalog all the nsSNPs expressed in the specific proteome. To achieve this goal, exome data were filtered for missense SNPs to use as a comparison. First, corneocytes were collected from the same general area with the use of five dermal patches. Afterward, these cells were extracted with sodium dodecyl sulfate and washed with sodium dodecanoate, before dithioerythritol reduction, iodoacetamide alkylation, and trypsin digestion were performed. The digested proteins then underwent downstream mass spectrometric analysis. Since information on genetic variation can flow from proteomic to genomic data sources and vice versa, both bottom up and top-down GVP discovery techniques were used separately. The generated datasets were aligned with proteomic databases for peptide and protein identification and analyzed in comparison with exomes for the presence and confirmation of GVPs.

From an initial analysis of four subjects, 84 GVPs have been characterized and validated by DNA sequence. The cumulative number of GVPs identified per individual ranged from 24 to 32. Most notably, 2 rare GVPs were found ( $MAF < 0.0001$ ). Without incorporating these, the application of the product rule led to a random match probability (RMP) of  $2.36 \times 10^{-06}$  in the European population. However, with the inclusion of the rare GVPs, the RMP decreased to  $1.63 \times 10^{-07}$ . Out of 336 inferences made, 10 were false positives. Recently, three more subjects have been evaluated. These datasets have produced 22 different GVP candidates, potentially bringing the total up to 106. The additional GVPs found could be the result of examining more individuals of a differing population. For each of the new individuals, the cumulative number of GVPs noted ranged from 26 to 31. Upon validation, the RMP is anticipated to decrease even further. Overall, the data depicts that proteomic processing of epidermal squamous corneocytes can lead to additional genomic content that is not always readily available at a crime scene. Going forward, a standard set of GVPs isolated and analyzed by this method needs to be determined. Thus far, GVPs corresponding to 13 genetic nsSNP loci have appeared in each subject. The reproducible results illustrate the potential use of this technique in the forensic science field. In the future, other tissue types can be assessed in the same manner.

#### Proteomics, Epidermal Squamous Corneocytes, Genetically Variant Peptides

### B118 The Determination of Biogeographic Ancestry Within Hispanic Populations

Cassandra Hernandez Setser, MSFS\*, Benbrook, TX 76132; Deanna S. Cross, PhD, University of North Texas Health Science Center, Fort Worth, TX 76107; John V. Planz, PhD, UNTHSC, Fort Worth, TX 76107; Ranajit Chakraborty, PhD, University of North Texas Health Science Center, Fort Worth, TX 76107

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the potential of single nucleotide polymorphisms (SNPs) to describe ancestry in such fine detail that it will separate closely related populations originating from Latin America.

**Impact on the Forensic Science Community:** This presentation will impact forensic science community by: (1) increasing their awareness and competence in a next generation technology that harnesses previously unused portions of the genome to obtain objective information on ancestry, and (2) allowing attendees to employ this technology in cases where standard genetic analyses are unable to provide useful information.

The STRs currently used in forensic genetics were specifically selected to have similar allele frequencies in all populations studied. However, there are instances when associating an unknown individual with a likely biogeographic ancestry (via loci with large differences in allele frequency) would be beneficial. When genotyping the Core 20 CODIS loci is not possible or when there is no CODIS hit, combining a number of ancestry informative SNPs together allows an examiner to detect the most likely ancestry associated with a sample and generate an investigative lead that is more objective than an eye-witness.<sup>1</sup> Therefore, it is hypothesized that a small panel of high quality SNPs is sufficient to differentiate closely related Hispanic populations into their specific biogeographic ancestry.

The previously existing Genomic Origins and Admixture in Latinos (GOAL) dataset was used computationally to develop such a panel.<sup>2</sup> Here, 164 unrelated samples from 5 different Hispanic populations were utilized to develop this SNP panel. Beginning with 897,336 polymorphisms genotyped within the population, PLINK was used to filter the dataset for linkage disequilibrium (LD), missingness (<10%), and minor allele frequency (>1%); 1215 SNPs were selected that had high  $F_{ST}$  values for the 4 pairwise comparisons with one country in common possible when studying 5 populations.<sup>3</sup> Further refinement was required when it became apparent that 71% of the 1215 SNPs could be attributed to Honduras, the only Central American population in this dataset. Therefore, only the ~10 SNPs with highest  $F_{ST}$  for the 1<sup>st</sup> and 2<sup>nd</sup> Country-in-Common (CiC) were selected for the final panel of 54 SNPs. This smaller, more balanced SNP panel achieved a similar degree of separation as the original 1215 SNP panel.

This ancestry SNP panel was compared to other SNP panels in the literature and tested for its predictive value. In comparison to the Kidd 55 and the Seldin 128, this panel of 54 SNPs produced a higher overall  $F_{ST}$  distribution.<sup>4,5</sup> Additionally, Bayesian method STRUCTURE and Principal Components Analysis method EIGENSOFT were employed to separate the populations.<sup>6,7</sup> However, it was found that this panel works best when employed as a 2-tiered analysis. The Honduras SNPs had a much higher mean  $F_{ST}$  than the other populations, so it is recommended that the panel be run for Honduras first, then for the other 4 countries. Discriminant Function Analysis (DFA) is being used to evaluate the panels to calculate centroids of each population for the prediction of unknowns.

In conclusion, this Hispanic Ancestry panel of 54 SNPs may prove useful when an individual is presumed to be Hispanic and more specific information is needed to generate an actionable investigative lead.

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#### Ancestry, Single Nucleotide Polymorphism, Population Structure



### B119 A Faster, Easier, and More Effective Bone Processing Method for DNA Analysis

*LeAnn Michelle Harrel, BS\**, Sam Houston State University, Huntsville, TX 77340; *Sheree R. Hughes-Stamm, PhD*, Sam Houston State University, Huntsville, TX 77340

**Learning Overview:** After attending this presentation, attendees will understand the potential for using a commercial DNA extraction kit and automated platform to screen and process samples from contemporary environmentally challenged bone samples to improve sample throughput and possibly reduce the need to outsource some casework. This paper proposes a method to quickly and efficiently extract DNA from bone samples without the need to pulverize bone tissue into a fine powder.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the benefits and limitations of demineralizing bone tissue without the traditional requirement for powdering. This sample processing approach is coupled with a commercial DNA extraction kit performed with and without automation for downstream short tandem repeats (STR) analysis.

In missing persons' cases, fire fatalities, mass disasters, and some forensic casework, skeletal samples are commonly used for human identification (HID) purposes. Bone and tooth samples are not routinely processed by all forensic laboratories, as the laboratory may not have the resources required such as bone grinding equipment, adequate lab facilities, or experienced analysts. Alternatively, specialized DNA analyses (e.g., mitochondrial analysis) may also be required. Due to the more complicated nature of these samples, skeletal remains may be sent to regional "hub" laboratories for processing. Traditional DNA extraction protocols involve the powdering of bone followed by a lengthy digestion (e.g., total demineralization) and DNA purification (e.g., organic or silica-based). While many laboratories that process skeletal remains prefer to process bone samples manually using their own in-house protocols, several commercial DNA extraction kits are available to standardize the process and improve sample throughput. However, these kits still require bone to be ground into a fine powder. This study explored the efficacy of a commercial DNA extraction kit and automated platform to purify DNA from small bone fragments to eliminate the need to crush the bone into a powder. This option has the potential to save time, reduce the risk of contamination, conserve evidence, and more effectively triage samples while also retaining the ability to automate the process (if desired).

In a pilot study, two main variables were evaluated to determine the most efficient protocol for whole bone chips using a commercial DNA extraction kit: chip number/size and incubation time. Three variations of each variable were tested in tandem for a total of nine combinations with five replicates each ( $N=45$ ). Bone fragments (50mg chips) were collected from two cadavers (buried and fire exposure). For controls, powdered bone (50mg) was tested in the same manner as the bone chips. Neither an increase in incubation time (2, 4, or 16 hrs) nor bone chip mass (50, 100, 150mg) and/or number (1 – 3 bone chips) significantly improved results compared to the current manufacturer's recommended protocol (one 50mg bone sample for 2 hrs). Therefore, results suggest that no further optimization of conditions would be required to successfully process bone chips (in lieu of bone powder).

To test the efficiency of an automated platform as a potential screening and/or processing tool for crime labs, twenty bones and five tooth fragments were collected from nine sets of skeletal remains that have been environmentally challenged (fire exposure, embalming, burial, and advanced decomposition). The results of this study show that although slightly less DNA was recovered from the whole bone chips and tooth fragments, STR success rates were comparable to the powdered samples.

Overall, this research has shown that eliminating the need to powder bone tissue can simplify the DNA extraction process without significantly reducing downstream STR success. The processing of bone chips also offers the unique possibility for further testing, as a second round of extraction can be performed on the remaining partially digested bone chips. Additionally, an automated extraction of bone chips could provide less-specialized labs a simple and affordable means of screening (or processing) skeletal remains in-house with their existing chemistry.

#### **Bone, Automation, Short Tandem Repeats**



### **B120 Forensic Match Probabilities and the Impact of Historical Migrations and Consanguinity: A Population Study**

*Ijaz Anwar, MPhil\**, School of Criminal Justice, Lausanne, Vaud 1015, SWITZERLAND; *Shahid Hussain, MPhil*, Centre of Excellence In Molecular Biology, Lahore 53700, PAKISTAN

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**THIS ABSTRACT WAS NOT PRESENTED.**



### B121 Developing a Contaminant-Abatement Method for Isolation DNA From Cancellous Bones

*Bryan Bernal, BS, Pearl River, NY 10965; Jordana Centauro, Westwood, NJ 07675; Tova Akerman, John Jay College of Criminal Justice, New York, NY 10019; Yilin Kong, HSD, John Jay College of Criminal Justice, New York, NY 10019; Richard Li, PhD\*, John Jay College, New York, NY 10019*

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**Learning Overview:** After attending this presentation, attendees will be familiar with the research that has been conducted for processing bone evidence for forensic DNA analysis. The method developed in this study shall produce a new approach for processing bone samples, particularly useful for processing cancellous bones (spongy bones).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by possibly affecting the time and efficiency in processing skeletal evidence.

Forensic DNA analysis of bone evidence is important in investigating a variety of criminal cases involving identification. However, bone samples must be processed prior to isolating DNA. It is necessary to remove the potential presence of biological materials from co-mingled remains. Additionally, environment-borne contaminants namely PCR inhibitors that interfere with forensic DNA analysis must be eliminated. Routinely, the outer surface of a bone fragment is cleaned using manual or mechanical abatement methods such as sanding. While these methods can be effective for removing contaminants from cortical bones (compact bones), they are not applicable to bone fragments that are largely cancellous bone materials. These methods can also be laborious when dealing with irregularly shaped bone fragments. Thus, developing a processing method for cancellous bones is highly desired.

This study is to address this issue and develop a simple method for processing cancellous bones as well as cortical bone samples prior to DNA isolation. A liquid-based technique was applied to the sample cleaning process prior to DNA isolation. The bone samples were cleaned by incubating them in a solution for a brief abatement of bone samples, thus removing potential contaminants. Since swine (*Sus scrofa*) bone is a useful model system for simulating human bones, swine bones were tested in this study. The microscopic studies suggested that the method is effective in removing small amounts of surface bone materials of cancellous bones. The effect of the contaminant-abatement method on the yields of DNA isolated was studied. Four trials of experiments were carried out. The average DNA yields of treated samples were slightly lower than that of untreated samples. A single-factor ANOVA was conducted to compare the yields of the samples. There was no significant difference in the yields of these samples. Next, the quality of DNA isolated was studied. Direct sequencing of amplified fragment at *Sus scrofa* mitochondrial *Cytb* locus was carried out. The electropherograms of the untreated control samples and treated samples were compared side-by-side. No adverse effect was observed among treated samples. This study demonstrated that this contaminant-abatement method of the bone fragments can be potentially useful for processing bone evidence, in particular for cancellous bones, prior to DNA isolation.

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#### Identification, DNA Isolation, Contaminant

### B122 A Developmental Validation of a Body Fluid Identification Multiplex Via DNA Methylation Analysis

Quentin T. Gauthier, MSFS\*, Hockessin, DE 19707; Sohee Cho, PhD, Seoul National University College of Medicine, Seoul 110-799, SOUTH KOREA; Bruce R. McCord, PhD, Florida International University, Miami, FL 33199

**Learning Overview:** After attending this presentation, attendees will understand the results of a developmental validation of a novel method for the determination of body fluids based on DNA methylation markers and sequencing techniques. The procedure involves a multiplex amplification of tissue specific differentially methylated regions followed by pyrosequencing to determine the identity of the tissue.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing results from the evaluation and developmental validation of a multiplexed approach to body fluid identification which is currently done in serology in several separate tests, expanding the information that can be gleaned from an individual's DNA.

The origin of a DNA extract can be used in criminal investigations to prove criminal intent and is possibly more compelling than simply confirming the presence of DNA. In the case of child abuse from a parent or guardian, it is the type of body fluid present and submitted as evidence that can aid investigators in determining the sequence of events, rather than the DNA's presence in the first place. The McCord DNA research group has located and validated several DNA methylation markers for identifying whether a DNA extract originated from blood, saliva, vaginal epithelia, or semen.<sup>1,2</sup> These loci, known as tissue specific differentially methylated regions (tDMRs) can show hypomethylation in one body fluid type and the opposite in other body fluids. Additionally, DNA methylation has been found to can predict biological age, but the body fluid can influence the accuracy of prediction models.<sup>3,4</sup> More recently, the authors combined the four best markers for these body fluids into a multiplex PCR assay and demonstrated their ability to still accurately identify the four aforementioned body fluids. However, questions remained about the ability for this technique to be implemented in to forensic laboratories.

In particular, the sensitivity of the method, the ability to identify mixtures of body fluids, and the effects of degradation and inhibition needed to be examined. Samples of saliva, blood, vaginal epithelia, and semen were collected, extracted by PCIA, and quantified by Alu primers. The samples then underwent bisulfite conversion to convert unmethylated cytosines to uracil. Samples were then amplified in multiplex and sequenced using a Qiagen Q48 Autoprep<sup>®</sup> Pyrosequencer (Qiagen, CA). For sensitivity, the multiplex was tested with input DNA from 20ng to 10pg. In the mixture study, the multiplex was tested with 100:0, 75:25, 50:50, 25:75, and 0:100 ratios of two body fluids at a time. For degradation, the samples were exposed to 95°C for 10-25 minutes, and for inhibition, samples were exposed to hematin and humic acid at concentrations of 0.08 mM and 0.24mg/mL, respectively.

The results of the sensitivity study showed the method working reliably with input DNA at 250pg, and with some results attained at DNA inputs all the way down at 50pg. For the mixture study, it was possible to make assumptions about which two body fluids were present, but not the exact ratio. The methylation value for a mixture of two body fluids was simply a value somewhere in between the expected methylation values of each body fluid on its own. For the degradation and inhibition study, the method continued to work for samples that were heated for up to 20 minutes, before negative effects were observed. For inhibition, the hematin and humic acid did not significantly affect the results when added to the sample before bisulfite conversion. However, if the inhibitor was added after bisulfite conversion, the sample would fail to amplify.

The overall results of this study have demonstrated this body fluid identification multiplex is able to accurately identify body fluids at concentrations that are largely like what is found in forensic cases. Additionally, the method works with mixtures of body fluids and withstand significant levels of degradation. Inhibition was a non-issue due to the bisulfite conversion process's action as a secondary cleanup of DNA extracts. This multiplex method to body fluid identification will open the door to more informative epigenetic tests in the near future. For example, age identifying methylation markers would give even more information to investigators.

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#### Body Fluid Identification, DNA Methylation, Epigenetics



### **B123 The Optimization of a Semi-Automated Differential Extraction Protocol for Recovery of Low-Level Male DNA From Sexual Assault Samples**

*Janelle Leo, BS\*, Philadelphia, PA 19150; Heather V. Milnthorp, MSFS, Center for Forensic Science Research & Education, Willow Grove, PA 19090; Heather E. McKiernan, MSFS, Center for Forensic Science Research & Education, Willow Grove, PA 19090*

**Learning Overview:** After attending this presentation, attendees will better understand the challenges associated with the extraction, isolation, and purification of sexual assault samples in which a low-level of male DNA is in the presence of an excess of female DNA. Attendees will also learn techniques for the successful recovery of purified male DNA using a combination of manual separation methods and automated extraction platforms to generate robust, highly-discriminatory STR profiles.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a highly efficient workflow solution for lower-throughput forensic laboratories to maximize the amount of easily interpretable discriminating genetic information which can be produced from sexual assault samples in which the male-to-female mixture ratio is in extreme proportions.

Often, sexual assault reporting is delayed, which can result in diminished quantities of male DNA being recovered off intimate swabs collected from the victim. This can subsequently lead to minimal success in the generation of an autosomal STR profile of the male perpetrator. With an excess of female DNA going into the extraction process, it is possible that not all the epithelial cells are being digested during the initial incubation. The residual intact or partially digested female DNA epithelial cells would then be pelleted with the sperm cells and carried forward through extraction and downstream analysis. Although Y-STRs may be a suitable alternative for these types of samples, application of this technology results in a decrease of discriminatory power in identifying the perpetrator.

The goal of the current research was to optimize the front-end manual separation of seminal and epithelial fractions, to be incorporated into a semi-automated differential extraction workflow using a robotic DNA extraction platform, with the end-goal of reducing the amount of female epithelial cells being carried over into the seminal fraction without increasing the amount of hands-on time required by the analyst. To do so, it was imperative to assess the differences in quantitative and qualitative performance of samples which underwent variable numbers of sperm pellet washes and to assess the effects of a single epithelial cell lysis step compared to two rounds of epithelial cell lyses. Preliminary data generated from vaginal swabs spotted with neat semen showed that increasing the number of wash steps and incorporation of a secondary epithelial lysis step both decreased the total quantity of human DNA recovered without negatively impacting the quantity of male DNA recovered in the seminal fraction.

The reliability of the previously discussed methods was also assessed with extreme male-to-female mixture ratios of 1:1000 neat seminal fluid to homogenized vaginal fluid. The number of wash steps was first assessed, which resulted in the addition of a second wash step decreasing the amount of total human DNA present ( $\bar{x}_1 = 0.0749$  ng,  $\bar{x}_2 = 0.0273$  ng), as seen previously. However, without visualization of a sperm pellet, the addition of a second wash step also drastically decreased the amount of male DNA present ( $\bar{x}_1 = 0.0703$  ng,  $\bar{x}_2 = 0.0183$  ng) and subsequently negatively affected the post-extraction male-to-female ratio, resulting in a more convoluted STR analysis. When paired with a single wash step, the incorporation of the secondary epithelial lysis step resulted in statistically significant differences in the amounts of total human and male DNA recovered from the seminal fraction compared to the usage of a single epithelial lysis step ( $F_{SA} = 11.723$ ;  $df = 1, 19$ ;  $p = 0.00303$  and  $F_Y = 15.563$ ;  $df = 1, 19$ ;  $p = 0.000949$ ), resulting in easily resolvable, highly discriminatory single source or male major contributor profiles.

This study demonstrates that increasing the number of wash steps applied to a sperm pellet during a differential extraction can be deleterious for sexual assault samples in which the male-to-female mixture ratio is in extreme proportions, due to loss of sperm fraction DNA during the additional wash steps. This study also demonstrates that utilization of a secondary lysis step is extremely beneficial to the production of isolated, highly-purified seminal fractions for further extraction utilizing a robotic extraction platform. Further work is being completed to assess any possible effects of the proposed method on analysis of the epithelial fraction as well as performance of the method with more extreme mixture ratios out to 1:5000.

#### **Differential Extraction, Method Optimization, Robotic DNA Extraction**



### **B124 An Examination of the Persistence of Indirectly Transferred DNA on Regularly Used Knives in a Stabbing Simulation**

Erica Cantor, BS\*, Indianapolis, IN 46227; Jessica Miller, BS\*, University of Indianapolis, Indianapolis, IN 46227; Krista E. Latham, PhD, University of Indianapolis, Indianapolis, IN 46227; Cynthia Cale, MS, Strand Diagnostics, Indianapolis, IN 46241; Gay L. Bush, PhD, Strand Diagnostics, Indianapolis, IN 46241

**Learning Overview:** After attending this presentation, attendees will have a better understanding of ways in which indirectly transferred DNA can potentially complicate the interpretation of relationships between DNA evidence and criminal acts. This presentation will: (1) demonstrate the persistence of transfer DNA on objects that have both important daily functions and potential evidentiary importance over the time-period of one week, and, (2) demonstrate the detection of both directly and indirectly transferred DNA on these objects over time.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a systematic investigation of transfer DNA persistence and the ways in which indirectly transferred DNA can complicate interpretations between DNA profile data and direct contact with evidentiary items. Advancements in DNA typing technologies have allowed for the generation of DNA profiles from smaller quantities of template DNA. As a result, the likelihood of amplifying DNA that has been transferred indirectly to an object has greatly increased. There are many variables that can affect the transfer and subsequent detection of DNA on inanimate objects.

This study expands upon previous research conducted by Meakin and colleagues that examined the persistence of secondary transfer DNA on inanimate objects.<sup>1</sup> In the current study, 12 participants were instructed to handle a knife for approximately one minute, twice a day, for two days to simulate regular daily use of an object. On the third day, the participants were instructed to shake hands with another participant for 10 seconds, pick up the knife and then stab the knife into a Styrofoam bowl repeatedly. Each knife was swabbed one hour and one week after the simulated stabbing event. DNA was purified from the swabs using the QIAGEN QIAamp DNA minikit, amplified using the GlobalFiler™ Polymerase Chain Reaction (PCR) Amplification Kit and analyzed on an Applied Biosystems® 3130xl genetic analyzer. The data was interpreted utilizing the Mixture Analysis Tool within GeneMapper® ID-X version 1.5. The DNA profiles identified were examined to address the following null hypotheses: (1) transfer DNA consistent with the handler will not be detected on knives sampled one hour after contact. (2) transfer DNA consistent with the handler will not be detected on knives sampled one week after contact. (3) a mixed DNA profile will not be detected in the samples collected one hour after handling. and (4) a mixed DNA profile will not be detected in the samples collected one week after handling.

DNA was detected on 91% of the knives sampled one hour and one week after handling. DNA mixtures consistent with the DNA profiles of both the knife handler and the person they touched were detected in most samples. Of the samples collected one hour after the stabbing event, 70% produced mixed DNA profiles. Of the samples collected one week after the stabbing event, 90% produced mixed DNA profiles. Additionally, extraneous DNA that could not be attributed to either participant in the pairing was detected in over half of the total samples, demonstrating the complex nature of DNA transfer.

The results of this study illustrate the potential issues and complexities regarding the interpretation of transfer DNA on regularly handled inanimate objects. In this study, DNA transferred by both direct and indirect means were detected on the objects examined. Additionally, only 10 seconds of contact between participant pairs was sufficient to indirectly transfer DNA from person to object via an intermediary individual. Therefore, this study illustrates the complex nature of DNA transfer and the difficulty in predicting the mode of DNA transfer (direct or indirect) based on the DNA profile identified on an object.

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**Transfer DNA, DNA Persistence, DNA Mixtures**





### B125 Nanoscale Imaging and Chemical Analysis of Extracellular DNA in Trace Biological Samples

Congzhou Wang, PhD, Virginia Commonwealth University, Richmond, VA 23284; Emily Brocato, Virginia Commonwealth University, Richmond, VA 23284; Christopher J. Ehrhardt, PhD, Virginia Commonwealth University, Richmond, VA 23284; Vamsi Yadavalli, PhD\*, Virginia Commonwealth University, Richmond, VA 23284-3028

**Learning Overview:** After attending this presentation, attendees will understand about high resolution microscopy approaches for characterizing cells and extracellular or "cell-free" DNA in biological samples. Attendees will learn how nanoscale morphological and mechanical data from forensically relevant cell types can complement information gained from traditional forensic tests.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing understanding of transfer mechanisms for DNA and/or cells and helping to create better strategies for the collection and preservation of DNA evidence in trace biological samples.

Several studies have shown that extracellular DNA (*eDNA*) can form a significant component forensic biological samples. For 'touch' or trace samples composed almost entirely of cells from the outermost layer of skin, *eDNA* may constitute a majority of total recoverable DNA. Yet, many biologically relevant aspects of *eDNA* and the relationship to co-deposited epithelial cells are not well understood. Detecting and mapping the extracellular DNA on epithelial cells, including the relative contributions of loosely-bound and surface-associated DNA could provide a unique look at its spatial/biochemical context. This has important implications for understanding the mechanisms of DNA transfer through touch, maximizing recovery of DNA from contact surfaces, and understanding its role in biological and forensically-relevant phenomena.

The goal of this study is to study the presence and relative quantity of *eDNA* on two forensically relevant epithelial cell types: non-keratinized buccal cells, and keratinized epidermal cells derived from the palm and fingers. Initially, the authors present images of these cell types taken with Atomic Force Microscopy (AFM) to understand their nanoscale topography. They then discuss "surface maps" showing the distribution of *eDNA* on individual cells. Maps across each cell type can be compared using samples obtained from different individuals. The prevalence of surface-associated *eDNA* was also examined across multiple sample washing steps to elucidate the nature of the attachment between the *eDNA* and the cell surface.

Results show that the presence and relative quantity of surface-associated *eDNA* can be analyzed on individual cells derived from both sources. The abundance of *eDNA* varied between the two cell types (~10%-16% for buccal and ~4%-9% for touch epithelial cells). Samples subjected to a water wash step show a significant decrease in the prevalence of *eDNA*, between ~20% and ~50%. This suggests that the corresponding proportion of DNA in each sample has non-specific physico-chemical interactions with the cell surface. To complement single cell experiments, we also investigated the effect of different buffer chemistries (e.g., water, EDTA, lysozyme) on the association of *eDNA* with 'touch' epidermal cell surfaces. Results suggest that the majority of *eDNA* (>80%) can be removed from the surface with a single water wash. A small fraction of *eDNA* (<5%), is removed from the cell surface after treatments with EDTA or lysozyme, suggesting that *eDNA* may be associated with the cell surface via cationic bridges or peptide interactions. Importantly, all the recovered fractions of DNA led to partial to full STR profiles that are comparable to profiles obtained through conventional extraction techniques, indicating the potential for faster, more efficient processing strategies for biological evidence.

#### Extracellular DNA, Atomic Force Microscopy, Trace DNA



### B126 Tracking DNA Loss in Forensic Touch Samples

*Jessica Tang, BS\*, Chicago, IL 60607; Ray Wickenheiser, MBA, New York State Police Crime Laboratory System, Albany, NY 12226-3000; Ashley Hall, PhD, University of Illinois at Chicago, Chicago, IL 60612*

**Learning Overview:** After attending this presentation, attendees will understand at which stages of the collection and extraction processes the highest loss of DNA from touch samples is observed, and the effects that different substrates can have on DNA yields.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying whether the initial collection of a touch sample by swabbing or an organic based extraction may benefit from changes to maximize the amount of touch DNA obtained. Additionally, knowing how touch DNA yields are affected by certain substrate types can guide future investigations into how to better deal with these surfaces.

The Locard exchange principle states that, with contact between two items, there will be an exchange of material—a concept central to the science of fingerprints. The hands are vectors for the transmission of forensic evidence; sweat and oil are transferred as the ridge detail that is the conventional information-bearing component of the exchange. However, for the last two decades forensic scientists have recognized the added capability of extracting “DNA fingerprints from fingerprints,” that is, the sweat and oil exchange contains a second information-bearing component in the DNA-containing cells that support genetic profiling: this is the science of touch DNA.

Touch samples contain a low quantity of DNA, and the manipulations of collection and analysis can further reduce this amount. In fact, studies have tracked up to a 90% loss of DNA during the performance of standard protocols. However, it has been not clear at which point(s) during collection and/or analysis the DNA is primarily lost. To better identify and define these points, three “mock fingerprint” controls were developed, each containing a specific, known quantity of DNA. To evaluate loss during collection and extraction, 20 µl mock fingerprints were pipetted: (1) on to a surface—to measure the quantity of DNA left after swabbing, (2) on to a swab—to quantify DNA retained on the swab, and (3) directly into the lysis buffer—to evaluate loss due to the manipulations of extraction. Samples were collected and analyzed using a baseline protocol—mock fingerprint deposition, collection with a cotton-tipped swab wetted with 2% SDS, organic extraction, and real-time PCR quantification.

Each of the three types of mock fingerprint controls were used to construct five-point standard curves (0-1500 cells, 0.00 – 9.00 ng DNA) by plotting DNA added vs DNA recovered after extraction. With the mock fingerprint controls, true fingerprints were deposited on three different surfaces—a brass door handle, a steering wheel, a glassine (drug) baggie—and then analyzed. The quantities of DNA extracted were plotted on the standard curve and values for the human DNA that had been present in the true fingerprints at deposition were interpolated. The values ranged from 3.38 – 5.39 ng, with recoveries of only 1.54% (0.0520 – 0.0830 ng) of the amount of human DNA originally deposited, with most of the DNA loss attributed to retention on the collection swab. Strategies to increase recovery based upon the data collected will be discussed.

#### **Touch DNA, Mock Fingerprint, Quantification**

### **B127 The Development of a Universal Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) Protocol for Differentiating a Mixture of Forensically Relevant Body Fluids Using microRNA (miRNA)**

*Zoe S. Garcia, BSc\*, Towson University, Towson, MD 21252; Cynthia B. Zeller, PhD, Towson University, Towson, MD 21252*

**Learning Overview:** After attending this presentation, attendees will understand the advantages of utilizing microRNA as a means of identifying forensically relevant body fluids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a simple and cost-efficient protocol that can not only be incorporated into the workflow of current forensic laboratories, but provide concrete serological results.

The protocol overall will be comprised of two parts. The first part demonstrates a proof of concept regarding individual miRNA extraction and visualization.<sup>1,2,4,5</sup> Extraction will be carried out with the GeneAll Ribospin II RNA purification kit under a polymerase chain reaction (PCR) hood that has been designated for handling RNA samples.<sup>1</sup> Samples will be pre-screened using the Nanodrop for the presence of sufficient amounts of RNA. A two-step assay will then be performed. The first step will use a universal stem-loop reverse transcription to form complementary DNA (cDNA) of mature miRNAs using the RevertAid H Minus First Strand cDNA Synthesis Kit from Applied Biosystems. Real-time PCR, or qPCR, will be implemented with hsa-miR-16a-5p- and hsa-miR-135a-5p-specific assays, found in blood and seminal fluid, respectively, through a universal PCR approach. Using a universal common reverse primer based off the stem-loop primer introduces a common probe that targets the 3' overhang.<sup>6</sup> Differentiation between the two miRNA sequences will depend on miRNA-specific forward primers. The results of the qRT-PCR (real-time reverse transcription PCR) of the samples will be analyzed in comparison to a house-keeping miRNA, U6 snRNA, to normalize the data via a C<sub>T</sub> comparative method. The two miRNA markers chosen will exhibit different percentages of guanines and cytosines within various sequences, or %GC content, and lengths, which allow differentiation through melt analysis. Initially, samples will be exposed to a 1<sup>st</sup>-derivative analysis through the Rotor-Gene Q.<sup>3</sup> Samples will then be visualized using the Rotor-Gene Screenclust High-Resolution Melt (HRM) Software to identify the specified markers based on their melting temperatures from their differing %GC contents and lengths.

After having proven that the miRNA markers can successfully differentiate the two body fluids, the second part of the protocol will continue. A mixture study will be performed using the same assay to determine if they both can be identified in the same sample in the following various aliquots (blood to seminal fluid): 1 to 1, 2 to 1, and 1 to 2. The aim is to be able to visualize differentiation of the miRNA markers using the Rotor-Gene Screenclust HRM Software, given that the markers have separate clusters. Subsequent research should investigate other miRNA markers for other forensically relevant body fluids, such as saliva.

#### **Reference(s):**

1. Grabmüller M., Madea B., Courts C. Comparative evaluation of different extraction and quantification methods for forensic RNA analysis. *Forensic Science International: Genetics* 16 (May 2015): 195-202. <https://doi.org/10.1016/j.fsigen.2015.01.006>
2. Hanson E., Lubenow H., Ballantyne J. Identification of forensically relevant body fluids using a panel of differentially expressed microRNAs. *Analytical Biochemistry* 387, no. 2 (April 2009): 303-314. <https://doi.org/10.1016/j.ab.2009.01.037>
3. Omelia E., Uchimoto M., Williams G. Quantitative PCR analysis of blood- and saliva-specific microRNA markers following solid-phase DNA extraction. *Analytical Biochemistry* 435, no. 2 (April 2013): 120-122. <https://doi.org/10.1016/j.ab.2012.12.024>
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**Forensic Science, miRNA, Body Fluids**



### **B128 The Generation of Interpretable, Single-Source, Short Tandem Repeat (STR) Profiles From Mixed Samples Using Optical Tweezers**

*Michael K. Valle, BS\*, Virginia Commonwealth University, Richmond, VA 23220; Nicole Auka, MS, Virginia Department of Forensic Science, Richmond, VA 23219; Joseph E. Reiner, PhD, Virginia Commonwealth University, Richmond, VA 23284-2000; Tracey Dawson Cruz, PhD, Virginia Commonwealth University, Richmond, VA 23284; Sarah J. Seashols Williams, PhD, Virginia Commonwealth University, Richmond, VA 23284-3079*

**Learning Overview:** The goal of this presentation is to show the robust nature of the optical tweezer for separating out target cells from mixed samples. Furthermore, after attending this presentation, attendees will see how a full single source STR profile can be generated from as few as 39 sperm cells separated from a mixed sample using the optical tweezer.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by exemplifying the utility of the optical tweezer for separating out target cells from a mixed sample to generate an interpretable, single source, DNA profile. When compared to the traditional method of differential extraction, this technique does increase the amount of time the DNA analyst spends on each suspected mixture sample. However, by implementing this technique on the front-end of the DNA analysis workflow, the DNA analyst spends less time on interpretation as a single source DNA profile is generated.

Optical tweezers are an ideal tool for cell separation and numerous reviews have described the operational principles behind single-focus optical tweezers. Although highly technical in nature, it suffices to say that an optical tweezer is a tightly focused laser beam in a low index medium (i.e., water,  $n=1.33$ ) that attracts dielectric particles having an optical index of refraction larger than the surrounding medium (i.e., cells,  $n \approx 1.36$ ). These dielectric particles or cells will migrate towards the focal spot of the laser beam and remain trapped for long distance transport (i.e., millimeters). To focus the laser beam to a sufficiently tight spot ( $D_{\text{spot}} \approx 1 \mu\text{m}$ ) the beam is sent through a high magnification (100x) immersion objective. This degree of magnification serves two purposes. The first is to create the highly-focused laser trap and the second is to enable microscopic cell identification by cell morphology or fluorescence. This technique provides a high degree of manipulation for cells trapped, since each cell can be moved in the x, y, and z direction. This property of the optical tweezer provides separation of the cells of interest from a mixed population.

Initially, optical tweezers were utilized to capture and remove defined numbers of sperm cells (5-60 cells) from neat semen samples. Quantification of the DNA resulting from these samples resulted in detectable, amplifiable DNA in qualitative agreement with expected results based on cell number. Full DNA profiles were developed in multiple replicates. Following the success of this preliminary work, three mixed populations of vaginal epithelial cells released from a cotton swab were mixed with diluted seminal fluid, and the optical tweezers were utilized to separate the spermatozoa from the mixed population.

The DNA was isolated from the tweezed sperm cells using the QIAamp DNA Investigator Kit (Qiagen™ NV), quantified with the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific), vacuum centrifuged and the entire extract was amplified using the AmpFLSTR® Identifiler® Plus Amplification Kit (Thermo Fisher Scientific). The amplicons were separated using the 3130xl Genetic Analyzer (Thermo Fisher Scientific) and all samples were analyzed with GeneMapper® Software Version 4.1 using a threshold of 50 RFU.

A total of nine samples ranging from 39 to 74 sperm cells were obtained via separation with the optical tweezer. Eight out of nine samples yielded full STR profiles, with only a single dropout allele in one sample.

In conclusion, this has shown that the optical tweezer is a viable instrument for target cell isolation and separation from mixed samples. Furthermore, the results of this research show the potential of the optical tweezer for generation of single source STR profiles from other types of mixed samples commonly found in forensic DNA casework.

#### **Optical Tweezer, Cell Separation, DNA Workflow**



### **B129 The Evaluation of the Short Tandem Repeat (STR) Genotype Concordance Between Massively Parallel Sequencing (MPS) and Capillary Electrophoresis (CE) STR Kits in Japanese Population Samples**

*Tetsushi Kitayama, PhD\**, National Research Institute of Police Science, Kashiwa, Chiba 277-0882, JAPAN; *Kevin Kiesler, MS*, Gaithersburg, MD 20899; *Lisa Borsuk, MS*, National Institute of Standards and Technology, Gaithersburg, MD 20899; *Becky Steffen, MS*, National Institute of Standards and Technology, Gaithersburg, MD 20899; *Takashi Fukagawa, PhD*, National Research Institute of Police Science, Kashiwa, Chiba 2770882, JAPAN; *Haruhiko Watahiki, MS*, National Research Institute of Police Science, Kashiwa, Chiba 2770882, JAPAN; *Yusuke Mita, PhD*, National Research Institute of Police Science, Kashiwa, Chiba 2770882, JAPAN; *Koji Fujii, PhD*, National Research Institute of Police Science, Kashiwa, Chiba 277-0882, JAPAN; *Peter M. Vallone, PhD*, National Institute of Standards and Technology, Gaithersburg, MD 20899-8314; *Natsuko Mizuno, PhD*, Kashiwa 277-0882, JAPAN

**Learning Overview:** After attending this presentation, attendees will understand the performance of Short Tandem Repeat (STR) typing kits by Massively Parallel Sequencing (MPS) and Capillary Electrophoresis (CE). Attendees will also understand the concordance rates comparing allele calls from MPS and CE STR kits in Japanese population samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reporting the concordance rates and sources of discordant of STR allele calls between MPS and CE STR typing kits.

In this study, the STR profiles were generated and compared for the purposes of generating population allele frequencies and understanding allele call concordance by both MPS and CE methods. For the population study, buccal swab samples were collected from 83 unrelated Japanese volunteers, and DNA was extracted using EZ1 Advanced XL with EZ1 DNA Investigator Kit. For the allele call concordance study, the 83 samples mentioned above and 39 samples selected from a larger set of 1,501 samples previously extracted from unrelated Japanese volunteers were used. Out of the selected 39 samples, 30 contained D19S433 silent allele (silent: GlobalFiler® PCR Amplification Kit; normal: PowerPlex® Fusion System).<sup>1</sup>

The MPS STR profiles were generated by Precision ID GlobalFiler® NGS STR Panel v2 with the Ion S5/S5XL/Chef System and ForenSeq DNA Signature Prep Kit with MiSeq FGx Forensic Genomics System and compared with the CE STR profiles generated by the GlobalFiler® PCR Amplification Kit and PowerPlex® Fusion System analyzed on a 3500xL Genetic Analyzer. The CE STR data from GlobalFiler® PCR Amplification Kit and PowerPlex® Fusion System were analyzed using GeneMapper® ID-X 1.4 software. The data from Precision ID GlobalFiler® NGS STR Panel v2 were analyzed using Converge Software v2.0 and HIDGenotyper plugin v2.0. The data from the ForenSeq DNA Signature Prep Kit were analyzed using ForenSeq Universal Analysis Software and STRait Razor v2s.

For population samples, there was no discordance observed in the allele calls generated by ForenSeq DNA Signature Prep Kit and CE STR kits except higher stutter and allele dropout caused by low peak height ratio found at some loci when using the ForenSeq DNA Signature Prep Kit. There were 6 discordances in 6 individuals at D2S441 locus between the Precision ID GlobalFiler® NGS STR Panel v2 and CE STR kits caused by a 1 base pair insertion just upstream of repeat region (9: Precision ID GlobalFiler® NGS STR Panel v2; 9.1: CE STR kits). There were 41 apparent discordances in 36 individuals at D10S1248, D12S391, and D1S1677 loci between the Precision ID GlobalFiler® NGS STR Panel v2 and CE STR kits that arose from bioinformatics issues found in the software and plugin.

The silent alleles at D19S433 were not amplified with Precision ID GlobalFiler® NGS STR Panel v2 as with the case of GlobalFiler® PCR Amplification Kit. On the other hand, the same “silent” allele at D19S433 was successfully amplified with ForenSeq DNA Signature Prep Kit as well as the PowerPlex® Fusion System.

Overall, MPS STR typing kits produced more discriminative information compared with CE STR typing kits. However, there were some bioinformatics issues on the reliability of the MPS STR analysis software that need to be resolved to produce full concordance to the known CE STR allele calls. The software updates of the algorithm may correct the discordances reported here in the near future.

#### **Reference(s):**

- <sup>1</sup> Fujii, K. et al., Typing concordance between PowerPlex® Fusion and GlobalFiler® based on 1501 Japanese individuals and the causes of typing discrepancies., *Forensic Sci Int Genet.*, 2016; 25:e12–e13.

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#### **Massively Parallel Sequencing, Short Tandem Repeat, Concordance**



### **B130 An Evaluation of the Stability of High Throughput Sequencing (HTS) of Microbial DNA From Compromised Human Samples**

*Francy Scarlett Nogales, BS\*, Virginia Commonwealth University, Richmond, VA 23223; Sarah J. Seashols Williams, PhD, Virginia Commonwealth University, Richmond, VA 23284-3079; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA 23284; J. Brooks, PhD, Virginia Commonwealth University, Richmond, VA ; Denise Wohlfahrt, BS\*, Virginia Commonwealth University, Richmond, VA 23284; Raquel Green, BS\*, Richmond, VA 23220; Antonio Tan-Torres, MS, Virginia Commonwealth University, Richmond, VA 23284; Kathleen D. Brim, BS, Henrico, VA 23294; Najai Bradley, Richmond, VA 23220*

**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the effects of the environment and contaminants to the use of High Throughput Sequencing (HTS) of microbial DNA from human samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information and validation concerning the use of microbial signatures in forensic applications.

The human microbiome study showed distinct microbial signature at different body sites that have the potential for development of forensic tools such as postmortem interval estimation method, human individualization, identification of trace evidence and human body fluid identification. Although microbial signature associated with each body fluid is distinct and can be utilized for the identification of body fluids at the crime scene, it is not known how this method will work for forensic evidence samples that are degraded (because of harsh environmental exposure) or contaminated (because of chemical treatments). This study would validate such techniques prior to implementation in the field. Although scientific research using HTS technology is an emerging field based on the cost-effectiveness, there is currently insufficient research regarding microbial signatures of compromised samples from human body fluids. This study aims to address that issue by assessing bacterial concentration, sequencing and classification of microbial taxa from body fluids frequently compromised or tampered at crime scenes in comparison to untreated samples.

To assess the effects of compromised samples through environmental exposure or chemical treatment, a variety of human body fluids were exposed to harsh treatment conditions that evidence samples could be subjected to at the crime scene. Venous blood, saliva, seminal fluid, urine, fecal matter, vaginal and menstrual secretions were collected from female and male volunteers under an approved human subjects research protocol. These were then exposed to prolonged times of 24 and 96 hours in high temperatures, ultraviolet exposure, bleach contamination and detergent exposure. Treated samples and positive controls were extracted using standard DNA isolation protocols and quantified using an optimized qPCR method with universal 16S rDNA primers. Amplification was conducted using the variable region V4 and sequenced on an Illumina® MiSeq FGx™ platform (Illumina®; San Diego, California).

The resulting data was analyzed via MiSeq® SOP Mothur version 1.36.1 and statistical comparison were obtained with analysis of molecular variance (AMOVA). The results from these analyses provide further evidence for the utility of microbial signatures for body fluid identification in forensic casework and assessed the error associated with this method.

#### **Microbiome, Body Fluid ID, Compromised Samples**



### **B131 The Development of Magnetic Carbon Nanotubes (Mag-CNT) for Dispersive Solid Phase Extraction (dSPE) of Cyanide Metabolite (2-Aminothiazoline-4-Carboxylic Acid) in Biological Samples**

*Sun Yi Li, BSc\*, Sam Houston State University, Huntsville, TX 77341; Jorn Chi-Chung Yu, PhD, Sam Houston State University, Huntsville, TX 77341*

**Learning Overview:** The purpose of this presentation is to familiarize the audience with the novel method of analyzing cyanide metabolite from biological samples using Magnetic Carbon Nanotubes facilitated Dispersive Solid Phase Extraction (Mag-CNT/d-SPE) coupled with Gas Chromatography/Mass Spectrometry (GC/MS) analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the application of the magnetic carbon Magnetic Carbon Nanotubes facilitated Dispersive Solid Phase Extraction (Mag-CNT/d-SPE) for the extraction of the cyanide metabolite, 2-aminothiazoline-4-carboxylic acid (ATCA), from biological samples. The use of this new method may help to confirm cyanide exposure in forensic analysis.

Approximately 8% of the cyanide exposure cases in 2016 were due to intentional poisoning. The lack of consistent and conclusive autopsy findings increases the difficulty in confirming cyanide exposure. Other than that, due to the reactivity and instability of cyanide, improper storage conditions of the biological samples further increase the unreliability of confirmation cyanide exposure by direct detection of cyanide. An alternative approach to confirm cyanide exposure is to detect its minor metabolite—ATCA. Current studies published to extract ATCA from biological samples mainly focused on the conventional solid phase extraction (SPE) or liquid-liquid extraction (LLE). In this work, the capability of Mag-CNT to extract ATCA from biological samples was investigated.

Magnetic carbon nanotubes (Mag-CNT) were first synthesized in-house. The capability of Mag-CNT to extract ATCA was first tested in water samples spiked with ATCA standard. In a microcentrifuge tube, 2 mg of Mag-CNT was added in 100  $\mu$ L of deionized water (D.I. water) and 1000 ng/mL of ATCA in triplicates. The samples were acidified with formic acid, vortex, and sonicated to extract for 10 min. The Mag-CNT were isolated with aid of a strong magnet and the supernatants were transferred in separate tubes. Back-extraction was performed on the Mag-CNT with 150  $\mu$ L D.I. water/ 5% ammonium hydroxide. The Mag-CNT were isolated again, and the back-extract were transferred to separate tubes. An isotopic compound, ATCA -  $^{13}\text{C}$ ,  $^{15}\text{N}$ , was used as the internal standard. The three portions (Mag-CNT, supernatant, back-extract) were dried under vacuum at 65°C and derivatized with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and subjected to gas chromatography/mass spectrometry (GC/MS) analysis. Optimization of the extraction parameters, including extraction time, amount of Mag-CNT, and types of Mag-CNT, was performed in synthetic urine and bovine blood.

The Mag-CNT is found to be capable of extracting ATCA from both biological samples. Average recoveries of ATCA were 97.28% and 99.19% for synthetic urine and bovine blood respectively. The new approach not only has a satisfactory bias and precision within  $\pm 20\%$  at the low, medium, and high concentration levels, but also has a quantitation limit of 30 – 1000 ng/mL that can detect endogenous ATCA level in human urine and blood. The novel methodology in extracting ATCA from biological samples has a potential of forensic application to assist in the confirmation of cyanide exposure and might serve as an alternative method to overcome some limitations associated with the conventional SPE and LLE methods.

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**Mag-CNT/dSPE, Cyanide Metabolite, ATCA**



### **B132 A Three-Year Review of Novel Psychoactive Substance (NPS) Prevalence in Drug Identification Casework**

*Keith-Dane H. Temporal, MS\*, NMS Labs, Grand Prairie, TX 75050-1215; Donna M. Papsun, MS, NMS Labs, Willow Grove, PA 19030; Frederick Strathmann, PhD, NMS Labs, Willow Grove, PA 19090; Barry K. Logan, PhD, NMS Labs/CFSRE, Willow Grove, PA 19090*

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**Learning Overview:** After attending this presentation, attendees will understand popularity trends in the United States since 2015 of Novel Psychoactive Substances (NPS) by using forensic drug identification casework and correlating that to trends from toxicology results from human performance and death investigation casework. The presentation will also enable attendees to anticipate potential future trends regarding NPS drug use in both seized drug and toxicological analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by better preparing forensic chemists and toxicologists to be able to detect and identify NPS in forensic studies and casework.

Increased NPS frequency in seized drug evidence often presages increased NPS positivity in death investigation casework. Typically, new substances are identified in seized drug casework before they are encountered in toxicology casework as the higher concentrations in seized material and the use of non-targeted screening methods, such as gas chromatography/mass spectrometry (GC/MS), enables more ready identification. Forensic toxicology methods, which must anticipate several matrices, require quantitative methods with lower detection limits, and the identification and detection of drug metabolites takes more time to develop and validate. When coupled with region-specific data, trends that link seized drugs and toxicology data can be useful information for entities addressing both the public health and public safety aspects of NPS use.

In the NMS laboratory, at least 92 unique NPS compounds were confirmed in seized drug casework via GC/MS analysis and confirmed by comparison with available reference standards, since September 2015. These lab cases were tallied and sorted by lab received date to generate trend data. Trends of NPS in drug identification cases were collected through at least three years of retrospective data-mining of historical LIMS data and were grouped generally by similar drug pharmacology: designer benzodiazepines, designer opioids, dissociatives, hallucinogens, stimulants, and synthetic cannabinoids.

Notable trends in this review include significant sudden increases in synthetic cannabinoid seizures (such as FUB-AMB and 5-Fluoro ADB) starting in mid-2016 with a noticeable decline by the end of 2016. For example, the total 5-Fluoro ADB detection rates in 2016 increased from 43 to 139 cases between the first and second quarter, peaked at 404 cases in the third quarter, and declined to 31 cases in the final quarter. Steady increases in designer opioid seizures (such as acetyl fentanyl and carfentanil) have been observed since 2016 and continue through mid-2018. Acetyl fentanyl, the most frequently detected designer opioid was reported in 21 cases in 2016, 62 cases in 2017, and 112 cases through the first half of 2018. Toxicological data from biological specimens within the same time-period were compared for correlation.

In conclusion, prominent trends in reported NPS cases were presented in a 3-year review of drug analysis data from a large reference lab. The implications of tracking prevalence and trends in drug seizure casework over time and in real time include providing valuable information for stakeholders in the forensic science community, controlled substance legislators, and national public health advocates as NPS recreational drug use inevitably evolves.

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#### **Novel Psychoactive Substances, Drug Analysis, Trends**





### **B133 The Forensic Evaluation of Cut and Torn Duct Tape Fractures: A Validation Study to Quantify the Quality of a Physical Match**

*Meghan Prusinowski\*, Morgantown, WV 26505; Tatiana Trejos, PhD, West Virginia University, Morgantown, WV 26506*

**Learning Overview:** After attending this presentation, attendees will understand the process for a physical evaluation of duct tape fracture edges and the features that are observed on the tapes. In addition, attendees will understand the developed method for reporting the quality of the fit of the tape edges and the statistical methods by which the reported scores are assessed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a quantitative method for assessing and reporting the quality of fit between known and questioned tape edges. This method and the use of score likelihood ratios offer additional support for examiners performing tape comparisons.

Duct tape is a common type of evidence found at multiple types of crime scenes such as sexual assaults, kidnappings, and bombings. Performing a physical examination has been shown to have high discrimination power between different duct tape samples. For similar tapes, the highest degree of association that can be determined is through a physical fit between two tape pieces. The conclusion of a fit between edges relies on the examiner's opinion to identify distinctive features across the tape ends. However, there are currently no standard methodologies and criteria for making those decisions. This study aimed to establish mechanisms to qualify and quantify tape end match features and evaluate if experimental data support the assumption that random tape physical fits are unlikely. Over 1,800 blind tape end comparisons (hand-torn and scissor-cut duct tape samples, stretched and pristine samples) were evaluated by two independent examiners and the occurrence of false positives and false negatives was calculated. The sets were utilized to assess the effects of the separation method and stretching on the examiner accuracy and distribution of match scores.

Moreover, a mock kidnapping case consisting of 288 end-tape comparisons was used to test the proposed models under extreme tape-stretching conditions. The victim was restrained and gagged with tape, and the tapes were severely stretched and folded during the sample recovery to simulate worst case scenarios. Match scores were systematically calculated as a relative ratio of observed matching sections per scrim area. The more distinctive features seen along the tape ends, the closer the match score was to 100%. Utilizing the frequency distribution of true positives and true negatives by match scores, score likelihood ratios were calculated. Score likelihood ratios represent a ratio of the probability of observing a match score given the fractured edges came from the same source versus the probability of observing a match score given the fractured edges came from different sources. The higher the score likelihood ratio value, the higher the probability the score resulted from a fracture pattern left after separation from the same source (~SLR 10-10,000) while lower scores resulted from tape ends originating from different sources (0.1 to <0.0001). Receiving Operating Characteristic (ROC) curves were used to visualize the sensitivity and selectivity of the methods. The overall accuracy of the examinations was 99.6% for hand-torn tapes and 99.8% for scissor-cut tapes, with 0% false positives and 1-2% false negatives. The calculated score likelihood ratios indicated strong support for the conclusion of a fracture fit when the match score was 80% or higher, and strong support for the conclusion of a non-fit when the match score was 25% or lower. Although extreme stretching of the tape in the mock case did not increase the false positive rates, it decreased the ability to identify true positives. The results of this study are anticipated to offer an effective strategy to qualify and quantify distinctive features in tape end examinations and to serve as a scientific foundation to develop standard methods that complement and substantiate the examiner's opinions.

#### **Duct Tape, Physical Match, Score Likelihood Ratios**

### B134 Chemical Waste From a Chemistry Forensic Laboratory and the Challenge of Segregating, Treatment, and Disposal

Marcus de Abreu Farias Costa\*, Instituto Laboratorial de Análises Forenses, São Luís, MARANHÃO 65080-805, BRAZIL; Natilene M. Brito, PhD, IFMA, São Luís, MARANHÃO 65030-005, BRAZIL

**Learning Overview:** The goal of this presentation is to show that chemical handling needs to be done carefully, attempting to complete the criminal investigation while cautioning to avoid accidents induced by incompatible chemicals and keep the environment preserved.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing procedures on handling chemicals to optimize the work, avoid accidents, and keep the environment preserved.

Because of rising crime and the necessity for effective criminal investigations through use of scientific evidence, more services have been required from the chemistry forensic laboratories. The effect of this reality is the volume growth of casework and chemical substances being used, resulting in a volume more and varied waste produced to discard. The added diversity and requirement for a short turnaround time to produce the report, require the consolidation of balanced methodology procedures to guarantee the worker health and security and environmental integrity. Therefore, caring for the produced waste such that it is segregated, disposed, and discarded with established procedures.

Although it could be found some research papers treating laboratory waste, the majority is directed to teaching and research laboratories in universities.<sup>1-4</sup> An abstract written by Adnan Lanjawi for the 18th International Conference on Occupational Health and Safety in 2016 about forensic chemistry laboratory waste was found. It describes that the author started a study on the waste management from the Dubai police forensic chemistry laboratory, addressing the chemical quantity, the labels, the storage space and equipment in use. One finding of the research was regarding chemical segregation stored in alphabetic order, rather than according to the waste's nature and properties.<sup>5</sup> Another article, published October 2nd, 2012, Vince McLeode reported in Forensic Magazine that, because of a leak in a discarded barrel with chemical residue, methanol and chloroform spilled to the basement of Pittsburgh criminal laboratory, in Pennsylvania state.

Considering the scarcity of research studies handling the appointed problem in criminal investigation laboratories, the human and environmental vulnerability and that U.S. and European countries account the largest fraction of forensic chemist employees, this research manifests itself as extremely important to the forensic science community.<sup>6</sup>

This work target is to make a survey of the routine exams achieved in a forensic chemistry laboratory in São Luís, Maranhão, Brazil, identifying the waste properties and to deal with their segregation, treatment and disposal. The consequences of negligence are that it may bring severe injuries to the laborers, environment and society, directly and indirectly, due to the products of forensic activity. The theories used to develop this research study were found in books, research papers, standard operating procedures of government forensic institutions and the law.

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4. GIOVANNINI, J. G.; TAVARES, G. A.; BENDASSOLLI, J. A. Avaliação das técnicas de precipitação química e encapsulamento no tratamento e destinação conjunta de resíduos líquidos contendo cromo e vidrarias de laboratório. *Química Nova*, v. 31, n. 3, mar. 2008. Disponível em: <<http://www.scielo.br/pdf/qn/v31n3/a35v31n3.pdf>>. Acesso em 10.06.2018.
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#### Chemistry Forensic Laboratory, Waste Production, Chemical Handling



**B135 WITHDRAWN**



### B136 The Determination of Weathering Levels of Gasoline Using Various Methods of Analysis

*Morgan N. Carpenter, BS\*, Sam Houston State University, Huntsville, TX 77340; William M. Davis, PhD, Houston, TX 77054*

**Learning Overview:** After attending this presentation, attendees will understand the value and limitation on the use of quantitative and/or qualitative analysis of weathered gasoline samples using various methods and their use in determining the extent of weathering in a gasoline sample.

**Impact on the Forensic Science Community:** This presentation will influence the forensic science community by looking at the possibilities of adding a metric to analyzing weathered gasoline samples in addition to observing the patterns that weathered gasoline samples display.

When suspicious fires are started with ignitable liquids, the evidence is partially or wholly consumed. The process by which the liquid evaporates is akin to weathering where the most volatile components of a mixture diminish in quantity by continuous exposure to the atmosphere. Weathering is temperature-dependent and results in a sequential loss of volatiles as a function of the component's vapor pressure at a given temperature.

Fire debris analysts are trained to recognize weathered ignitable liquid residues and consider it when classifying a detected substance. The fire debris community relies upon the ASTM E1618 standard that requires reference liquids and reference chromatograms in a case file. A ratiometric approach is proposed to estimate weathering of the most common ignitable liquid, gasoline. The integration ratios of target compound peaks by gas chromatography with mass-selective detection (GC/MS) could be a starting point.

Five gasoline samples were examined, all summer blends from the Houston area, that were weathered at 20°C under a continuous stream of nitrogen to extents ranging from 10% to 90% by increments of 10%. The samples were then analyzed by GC/MS. Five sets of data were collected for each of five brands. Integration results for 11 target compounds (2,2,4-trimethylpentane, toluene, ethylbenzene, o-xylene, m-xylene, p-xylene, 1,2,4-trimethylbenzene, 1,2,4,5-tetramethylbenzene, naphthalene, 2-methylnaphthalene and 1-methylnaphthalene) were tabulated. Intra-sample integration ratios were then calculated for each compound. These ratios were then compared with the observed inter-compound ratios between unweathered exemplars. Finally, the latter ratios were averaged over the five brands.

In all gasoline samples that were weathered to  $\geq 80\%$  the two moieties with the highest room temperature vapor pressures, 2,2,4-trimethylpentane and toluene, were undetected. In other samples, ethylbenzene was undetected at 90% weathering. Another noticeable feature is that all ratios remain relatively unaffected when weathering is less than 50%. For all gasoline samples, the two compounds with disparate vapor pressures (o-xylene and 1-methylnaphthalene) were detected throughout. The inter-compound ratios of 2-methylnaphthalene to o-xylene ranged 1 to 9 and followed a complex polynomial function ( $n=6$ ). A drawback in this technique is the variance in the formulations of the gasoline. O-xylene to 1-methylnaphthalene ratios range from about 5:1 to 20:1 making estimates unreliable. The most consistent ratios are those between o-xylene and 1,2,4-trimethyl benzene (0.43-0.47). However, given the similarity in vapor pressures the variation as a function of weathering is less dramatic.

A second approach was investigated where integrated results for the ten most abundant compounds were converted to pseudo-mole fractions and then used to calculate their combined contribution to the vapor pressure using Raoult's Law. Again, small changes are observed up to 50% weathering.

#### Fire Debris, Arson, Weathered Gasoline



### **B137 Drug Trends in Correctional Facilities: An Assessment of Forensic Drug Chemistry Casework**

*Mollie Mares, MSFS\*, NMS Labs, Willow Grove, PA 19090; Frederick Strathmann, PhD, NMS Labs, Willow Grove, PA 19090; Barry K. Logan, PhD, NMS Labs/CFSRE, Willow Grove, PA 19090; Francis X. Diamond, BS, Willow Grove, PA 19090*

**Learning Overview:** After attending this presentation, attendees will be able to discuss drug trends observed in a correctional facility's seized drug casework, with particular focus on the trends of synthetic cannabinoids and other controlled substances in the Mid-Atlantic region.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness among forensic scientists of the trends in controlled substances identified in exhibits received from correctional facilities from September 2015 to June 2018.

Both peer-reviewed literature and media reports have suggested that there is an increasing use of synthetic cannabinoids and other NPS drugs in correctional facilities in several countries. Possible explanations for their popularity are their potency and the ease with which they can be smuggled into these facilities, together with the fact that they will not be detected in routine drug screening procedures. Synthetic cannabinoids are typically powders, but because of their potency, solutions of the drug can be sprayed onto plant-like material or increasingly onto paper for smuggling and ingestion. Use of synthetic cannabinoids in correctional facilities is a serious public health issue, due to the effects of the drug, which can include aggression, agitation and dissociation that can result from the consumption of the drugs along with more serious life-threatening effects of seizure, arrhythmia, and death.

The goal of this project was to determine if there were trends in the type of substances submitted from correctional facility-type locations submitted to our laboratory. Example locations include correctional facilities, prisons, and jails. Another aim of the project was to determine if the observations made were localized to particular facilities or if they were seen across multiple facilities, counties, and states. Data for this project was obtained from the Novel Psychoactive Substances (NPS) program at NMS Labs. The data from the NPS program was compiled from forensic chemistry casework and was extracted from Laboratory Information Management System (LIMS) at NMS Labs. The results obtained for this research came from data obtained using gas chromatography/mass spectrometry (GC/MS).

Compilation of the data resulted in correlating trends in the Mid-Atlantic region. From a state in the Mid-Atlantic region, synthetic cannabinoids were confirmed in 127 out of 3,468 cases (3.7%) submitted to the NMS laboratory. Fifty-five percent (55%) of those cases containing synthetic cannabinoids came from seven correctional facilities. Looking specifically at those correctional facilities, 5-Fluoro ADB, FUB-AMB (AMB-FUBINACA), and ADB-FUBINACA were the top synthetic cannabinoids confirmed. The trend of synthetic cannabinoids observed from correctional facilities in this state correlates to the findings from non-correctional facility submissions in this state as well. Of the non-synthetic cases submitted to the correctional facilities in this state, the other major compounds confirmed were marijuana, THC (Delta-9-tetrahydrocannabinol), hashish, and PCP (Phencyclidine).

Looking at a different state in the Mid-Atlantic region, 92 cases came from one county correctional facility. At this county correctional facility, 5-Fluoro ADB, FUB-AMB (AMB-FUBINACA), and ADB-FUBINACA were also the top synthetic cannabinoids confirmed. Looking at that county as a whole, those same synthetic cannabinoids were the top synthetic cannabinoids observed in the county. Outside of synthetic cannabinoids submitted to this correctional facility, approximately half of the non-synthetic submissions were pharmaceuticals, with buprenorphine and naloxone being the top pharmaceuticals being confirmed. Some of the other non-synthetic compounds that were confirmed included heroin, methamphetamine, alprazolam, fentanyl, and cocaine.

From September 2015 to June 2018, it was observed that 5-Fluoro-ADB was the highest synthetic cannabinoid seen in forensic chemistry drug casework submitted to our laboratory nationally, as well as in the specific correctional facilities investigated in the Mid-Atlantic region. In conclusion, trends in synthetic cannabinoids in correctional facilities have similar trends to the synthetic cannabinoids confirmed in non-correctional facility casework. These trends and observations are not localized to a single facility but seen in multiple facilities across state and county lines. The synthetic cannabinoids confirmed in correctional facility cases correlate to the synthetic cannabinoids seen in forensic drug chemistry casework overall that was submitted.

#### **Correctional Facilities, Synthetic Cannabinoids, Controlled Substances**



### **B138 Differentiation and Identification of U-47700, U-51754, and U-49900 Using Gas Chromatography/Mass Spectrometry (GC/MS) and Nuclear Magnetic Resonance (NMR) Spectroscopy**

Krystal T. Byrd, BSc\*, Fort Washington, MD 20744; Susan M. Schelble, PhD, Metropolitan State University of Denver, Denver, CO 80217; Kelly M. Elkins, PhD, Towson University, Towson, MD 21252

**Learning Overview:** After attending this presentation, attendees will understand methods to differentiate and identify emerging synthetic opioids U-47700, U-51754 and U-49900 using gas chromatography/mass spectrometry (GC/MS) and nuclear magnetic resonance (NMR) spectroscopy, including the influence of salt forms and solubility.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating methods to rapidly analyze emerging synthetic opioid new psychoactive substances (NPS) "legal high" compounds.

U-47700 (*trans*-3,4-dichloro-*N*-[2-(dimethylamino)cyclohexyl]-*N*-methyl-benzamide, 329.3 g/mol), also known as "Pink", "Pinky", or "U4", is a synthetic opioid known to be 7.5 times the potency of morphine and binds the mu opioid receptor. It has been documented in cases to cause adverse effects such as respiratory depression, cyanosis, pinpoint eyes, and tachycardia. U-47700, which gained popularity in U.S. forensic laboratories in 2015, was placed into a 24-month emergency action Schedule I by the Drug Enforcement Administration (DEA) in 2016 after a surge of deaths were confirmed as linked to it. Surrounding this scheduling, two structural analogs of U-47700 appeared in the drug market, U-51754 (*trans*-3,4-dichloro-*N*-[2-(dimethylamino)cyclohexyl]-*N*-methyl-benzeneacetamide, 343.3 g/mol) and U-49900 (*trans*-3,4-dichloro-*N*-[2-(diethylamino)cyclohexyl]-*N*-methyl-benzamide, 357.3 g/mol). These newer synthetic opioids have not been scheduled and very little literature or data is available. U-51754 is described by users as more dysphoric and dissociating than U-47700. However, key information is missing about both U-51754 and U-49900 including GC retention times, MS fragmentation patterns and NMR spectra. While current methods primarily focus on the detection of traditional opioids in drug-related overdoses the ever-adapting drug market makes it imperative for an increase in optimal methods that will detect, differentiate, and identify the emerging synthetic opioids.

1D and 2D NMR spectra for U-49900 are available in a recent publication.<sup>1</sup> <sup>1</sup>H NMR spectra (32 scans) were collected on a 400 MHz JEOL SS NMR spectrometer for each synthetic opioid (5 mg commercial standard) dissolved in 0.7 mL of deuterated chloroform. A mixture of U-49900 and U-51754 was created using 0.150 mL and 0.300 mL, respectively, of the above samples. U-51754 and U-49900 differ in structure: U-51754 has a dimethyl substituent group on the cyclohexylamine and U-49900 has a diethyl substituent group in that position. U-51754 also has a methylene separating the chlorinated benzene ring from the amide bond than can be used to differentiate it from U-47700. These differences are reflected in the NMR spectra and can be used to identify the components of the mixture: U-51754 exhibited a singlet at 1.7 ppm attributed to its cyclohexylamine dimethyl groups and U-49900 exhibited a quadruplet at 2.65 ppm and 3.4 ppm attributed to the CH<sub>2</sub> in its diethyl groups and a triplet at 1.0 ppm corresponding to the CH<sub>3</sub> of the cyclohexylamine ethyl group. GC/MS spectra of each substance and separation of a mixture of the three synthetic opioids will also be presented. UV-Vis and ATR FT-IR spectra of the three compounds will also be presented.

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**U-47700, U-51754, U-49900**



### B139 Fingerprint Recovery Methods From Submerged Knives in Different Aquatic Environments

Emily L. Horrocks, MSc\*, University of Portsmouth, Ravelin House, Portsmouth, Hampshire PO1 2QQ, UNITED KINGDOM

**Learning Overview:** The goal of this presentation is to demonstrate methods to recover fingerprints from different types of wet knives and how the aquatic environments affect the fingerprints.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) showing how to get fingerprint recovery from wet items, (2) increasing awareness and understanding of how the texture and environment influence the chosen method, and (3) highlighting the importance of comprehensive research to give investigators and practitioners confidence in the method they have selected to process each wet item.

There are many challenges when visualizing and recovering fingerprints of evidential value from submerged items. This is due to many variables, including the effect of water.<sup>1</sup> Some believe bodies of water to be a destructive environment, however research has shown that fingerprints can survive.<sup>2-4</sup> Moreover, it is the method used that can affect the quality of the recovered fingerprint. There is little research looking at the survivability of fingerprints in aquatic environments and fewer still researching the effect of the environments on the marks.<sup>5</sup>

With the increase in knife crime in the United Kingdom (UK), it is imperative that the best method for each crime scene is investigated and thoroughly researched. In England and Wales, from April 2016 to March 2017, there were 34,700 reported offenses involving a knife or sharp object. This is the highest reported number of incidents since 2011 and a 20% increase on 2016, showing a big increase in knife crime, making this a prominent issue for Police Forces.<sup>6</sup> Bodies of water are common in the UK, with a mix of rivers, ponds, lakes, estuaries, and the sea, making them an ideal place to dispose of a weapon. It is important to understand the environment the substrate was found in and how this can affect not only the fingerprint but the substrate itself.

This study focuses on identifying methodologies that have not been fully considered and tested for recovering fingerprints from knives submerged in different aquatic environments. It examined the effect of different bodies of water, sea, river and harbor, and the texture of the substrate on the development and visualization of the mark. The aim was to help improve the effectiveness of forensic methods for underwater investigations, and to provide a guide for police and forensic practitioners on the evidence potential of items found in water. However, this work is on-going in these preliminary studies; a variety of fingerprint development methods were used, and different sequential processes were evaluated to optimize the approach for each water type and each knife.

In this study, the clearest marks were developed using cyanoacrylate fuming from the sea water. However, previous research had shown “salt water” to be more destructive and the UK Home Office manual does not recommend cyanoacrylate fuming for wet items.<sup>7,8</sup> This result highlights the need for further research into what is affecting the efficiency of cyanoacrylate fuming.<sup>9</sup> Each water type produced varying results that brings into question the influence the environment has. This study shows that water does not remove fingerprints and there is a need to identify the best method for each environment and substrate. These results have significant impact on forensic practice and increase awareness for investigating underwater crime scenes in the future.

#### Reference(s):

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2. Somaya Madkour et al., “Development of latent fingerprints on non-porous surfaces recovered from fresh and sea water,” *Egyptian Journal of Forensic Sciences* 7, no. 3 (July 2017): 1-12.
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5. Raul Sutton, Claudio Greci and Lucie Hrubesova, “A Comparison on the Longevity of Submerge Marks in Field and Laboratory Conditions,” *Journal of Forensic Identification* 64, no. 2 (2014): 143-156.
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8. Home Office Centre for Applied Science and Technology (CAST), “Fingerprint Visualisation Processes” in *Fingerprint Visualization Manual*, ed. Helen Bandy (Home Office, 2014), 2.3.12.
9. Matej Trapecar, “Finger marks on glass and metal surfaces recovered from stagnant water,” *Egyptian Journal of Forensic Sciences* 2, no. 2 (June 2012): 48-53. Knives, Underwater, Fingerprints

#### Knives, Underwater, Fingerprints



### **B140 The Determination of a Canine Olfactory Detection Threshold Using a Universal Detection Calibrant (UDC)**

*Kelvin J. Frank, Jr., BS\*, Florida International University, Miami, FL 33174; Rodolfo Mesa, BS\*, Miami, FL 33174*

**Learning Overview:** The study essentially attempted to determine a possible canine odor detection threshold using a dose response curve. Results from approximately 40 canines tested will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing attendees to gather more insight into the development of a UDC for canines and provide detailed information on its use and benefits to the canine community. This topic will help the forensic community gain a more in depth understanding of canine olfactory thresholds and how this plays an important factor in their roles.

The current study investigated the response of biological detectors to varying odor levels presented by the UDC. Variations of the odor levels was accomplished using a Controlled Odor Mimic Permeation System (COMPS) developed by the International Forensic Research Institute (IFRI) at Florida International University. Under this system, the target odor is housed in a permeable material which releases the identified odor at a known and controlled rate. The permeation rate of the 1-Bromooctane was altered by adjusting the parameters of the permeable material. The rate was measured using gravimetric analysis as well as headspace solid phase micro-extraction (HS-SPME). Permeation rates were reported in mass as a function of time. The goals of this study were to decrease the odor availability/permeation rate of the Universal Detection Calibrant (UDC) and observe the percentage of positive canine alerts.

Biological detectors such as canines, unlike analytical laboratory instruments, have no procedure to ensure that they are working satisfactorily daily. This has led to the current development of a Universal Detection Calibrant (UDC) training aid at the International Forensic Research Institute (IFRI) at Florida International University. The UDC, much like an analytical instrument calibrant, allows canines to be calibrated to ensure that the canine is performing within acceptable working limits. This would allow for additional documentation of the canine's daily working parameters in addition to routine training records. In addition, comparison among different canine detection categories such as Drugs, Explosives, guns, cadavers will now be possible. Depending on the discipline, a canine may require greater sensitivity to the odor(s) it is trained on. Cadavers for example, must detect trace amounts of human odor and theoretically should have a lower detection threshold.

Prior analysis of several compounds revealed 1-Bromooctane (1-BO) as the compound with the greatest potential to be used as the biological calibrant. This compound was selected based on several criteria. The first determined the ease and affordability of the compound. The second investigated the health hazards to both canine and handler. Compounds were further selected if they possessed no special handling or disposal requirements. Scarcity of compound in the natural environment was also examined to ensure that compound was not a current target odor for canines.

The current study investigated the response of biological detectors to varying odor levels presented by the UDC. Variations of the odor levels was accomplished using a Controlled Odor Mimic Permeation System (COMPS) developed by IFRI. Under this system, the target odor is housed in a permeable material which releases the identified odor at a known and controlled rate. The permeation rate of the 1-Bromooctane was altered by adjusting the parameters of the permeable material. The rate was measured using gravimetric analysis as well as headspace solid phase micro-extraction (HS-SPME). Permeation rates were reported in mass as a function of time.

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#### **Universal Detection Calibrant, Permeation Rate, COMPS**





### B141 The Detection of Arson Accelerants Using Porous-Layer Open-Tubular (PLOT) Cryoadsorption

*Daniella M. Hernandez\**, Littleton, CO 80127; *April A. Hill, PhD*, Metropolitan State University of Denver, Denver, CO 80217

**Learning Overview:** After attending this presentation, attendees will gain an understanding of: (1) the basic principles and practice of Porous-Layer Open-Tubular (PLOT) Cryoadsorption as a sample preparation technique, and (2) the advantages of using PLOT-Cryoadsorption in place of carbon strips for the extraction of accelerant residues.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that, compared to the conventional method of extraction using carbon strips, arson samples extracted using PLOT-Cryoadsorption yield higher signals and lower background when analyzed by gas chromatography/mass spectrometry (GC/MS).

Arson crimes have an extremely low conviction rate due, in part, to the challenge of isolating and identifying the small amount of accelerant residue that remains after a fire is extinguished. Most techniques currently used to collect accelerant residues are based on headspace sampling vapors (i.e., accelerant) in the headspace above suspected arson evidence are collected onto adsorbent media such as activated charcoal. The primary disadvantage of these techniques is that they often require hours to collect a detectable amount of accelerant residue. A new technique for the headspace sampling of accelerant residues, porous-layer open-tubular cryogenic headspace sampling (PLOT-Cryoadsorption), is being investigated. In PLOT-Cryoadsorption, suspected arson evidence is placed in a sealed container and heated in a modified gas chromatograph (GC) while a flow of helium is applied to sweep the resulting headspace gasses from the sample container into a PLOT capillary. This PLOT capillary, which is simply a short section of gas chromatography column, is housed in a cryogenic chamber held at low temperature to trap the analytes. The analytes are then desorbed by passing a solvent through the PLOT capillary and collecting it in a vial for analysis by gas chromatography-mass spectrometry (GC/MS). In the current study, PLOT-Cryoadsorption was compared to the conventional method of isolating accelerants by heating suspected arson evidence in a sealed container containing an activated carbon strip. Mock arson samples were prepared by spiking 1-inch squares of clean cotton rag with 250  $\mu\text{L}$  of accelerant (kerosene, gasoline, or diesel). The mock samples were analyzed after burning for varying lengths of time ranging from 0 seconds (neat samples) to self-extinction at  $\sim 1$  minute. Duplicate samples were extracted using carbon strips and PLOT-Cryoadsorption to allow for direct comparison of the two techniques. The ASTM standard for analysis of arson samples by carbon strip extraction recommends heating for anywhere from 30 minutes to 16 hours. In this study, the carbon strip extractions were performed at  $60^\circ\text{C}$  for 5, 15, 30, 60, and 120 minutes. The PLOT-Cryoadsorption extractions were carried out at  $125^\circ\text{C}$  for 0.5, 1, 5, 10, and 15 minutes. Across the board, the chromatograms for residues collected using PLOT-Cryoadsorption gave higher abundances and lower background signals compared to those collected using carbon strips. Further, PLOT-Cryoadsorption gave distinct accelerant residue patterns in little as 30 seconds of extraction time. The results indicate that the use of PLOT-Cryoadsorption in arson analyses could allow for the detection of accelerant residues in less time and with greater precision than that achieved using the current method of activated carbon strips. Hence, PLOT-Cryoadsorption could help drastically improve the rate of arson convictions.

**Arson, Accelerants, PLOT-Cryoadsorption**



### **B142 Automatic Groove Identification in 3D Bullet Land Scans Using Statistical Models**

*Nathaniel M. Garton, MSc\*, Center for Statistics and Applications in Forensics, Ames, IA 50010; Heike Hofmann, PhD, Center for Statistics and Applications in Forensics, Ames, IA 50011; Ulrike Genschel, Center for Statistics and Applications in Forensic, Ames, IA 50011*

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**Learning Overview:** After attending this presentation, attendees will understand the principles behind some of the data preprocessing steps in bullet matching algorithms. Attendees will also see how the implementation of statistical models in the data preprocessing steps lead to increased matching accuracy in the overall matching algorithm.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the accuracy and reliability of automated bullet matching algorithms.

Automated comparison of bullet marks is an important area of current research. Success in devising such a procedure will result in robust tools and reproducible analyses for quantifying the strength of matching evidence between bullets. Recent work by Hare, Hofmann, and Carriquiry utilizing data generated from high-resolution 3D scans of bullet lands has shown promising results towards this goal.<sup>1</sup> In this work, the authors demonstrated the utility of statistical learning techniques for automatically comparing bullet marks and probabilistically quantifying the strength of matches between bullets. Steps involved in the preprocessing of the data are known to be integral to the success of the overall algorithm. One such step in the procedure requires identifying and removing data from the groove engraved area of the scan. Automation of the groove identification process is necessary for an end-to-end bullet matching algorithm, and while significant progress has been made towards reliable automatic groove identification, aspects of this procedure leave open the possibility that either more data are removed than is necessary, or the groove engraved area is not entirely removed. In either case, a loss of accuracy is expected. In pursuit of a more principled approach to identifying groove locations in these scans, the authors propose the use of a specific type of statistical model which combines ideas from statistical nonparametric function estimation and changepoint analysis to estimate the number and location of grooves in a 3D bullet land scan in a statistically rigorous way. Changepoint analyses have classically been used on processes changing over time to detect when some aspect of that process has changed. Examples of such applications are often found in finance, where the goal might be to detect a meaningful or anomalous fluctuation in the stock of a given company and when such a fluctuation occurred. The model results in a likelihood which is piecewise constant as a function of the changepoints, rendering maximum likelihood estimation infeasible. Thus, the authors opt for a Bayesian approach and estimate the groove locations via Markov chain Monte Carlo methods.

The authors will demonstrate the effectiveness of the proposed method on actual bullet land scans and compare the results to the current methods of groove identification. They also will explain how their procedure might be improved upon further.

**Reference(s):**

- <sup>1</sup> Hare, Eric, Heike Hofmann, and Alicia Carriquiry. 2017. "Automatic Matching of Bullet Land Impressions". *The Annals of Applied Statistics* 11, no. 4 (December):2332–2356.

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**Groove, Identification, Bullets**



### **B143 The Estimation of True Compositions of Volatile Compounds in Headspace Via Solid Phase Microextraction (HS/SPME) and Inverse Gas Chromatography (IGC)**

*Joonyeong Kim, PhD\*, Buffalo State College, Buffalo, NY 14222*

**Learning Overview:** The goal of this presentation is to demonstrate a strategy of the estimation of true compositions of volatile compounds in headspace via solid phase microextraction (HS-SPME) and inverse gas chromatography (IGC). The compositions of volatile compounds in headspace (HS) analyzed by a solid phase microextraction (SPME) sampling method usually differ from true compositions mainly because the partition coefficients for these compounds between headspace and a SPME fiber are not equal. In this presentation, the author reports an analytical strategy to more accurately quantify relative compositions of selected hydrocarbons present in gasoline residue by employing inverse gas chromatography (IGC) coupled with a HS-SPME sampling method.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing a way to estimate true compositions of target compounds in headspace as well as solid substrates using partition coefficients obtained via inverse gas chromatography (IGC). The results produced in this work and future studies can be applied to the quantification of target volatile/semi-volatile compounds in various forensic samples including fabric, cardboard, and carpet.

Headspace concentration method, coupled with solid phase microextraction (HS-SPME), provides an easy and convenient sampling method for analyzing volatile/semi-volatile compounds such as gasoline residues present in solid samples. Compared to traditional sampling techniques such as passive headspace concentration with activated carbon and subsequent solvent extraction, it is a solvent-free sample preparation technique that integrates sampling, isolation, and concentration with enhanced sensitivity. Its simplicity of use, relatively short sample processing time, and fiber reusability have made SPME an attractive choice for many forensic analytical applications.

Despite its successful sampling from various solid substrates, a quantitative analysis of target compounds present in solid substrates via HS-SPME cannot be done in a straightforward manner. It is primarily because SPME fibers are not evenly sensitive to all target compounds in headspace. In addition, all compounds present in solid substrates do not equally diffuse into headspace. Thus, the relative peak areas in chromatograms via HS-SPME do not properly represent the true compositions of all compounds present in solid substrates. Both partition coefficients of volatile compounds at headspace/SPME and headspace/solid substrate are required to obtain true compositions of target compounds present in the solid substrate via HS-SPME.

In this work, an analytical strategy to more accurately quantify relative compositions of selected hydrocarbons present in three model headspace systems using estimated partition coefficients between headspace and a PDMS is presented. First, inverse gas chromatography measurements using a column packed with a solid support coated with polydimethylsiloxane (PDMS) were conducted to obtain the thermodynamic and chromatographic data needed for the estimation of the relative partition coefficients for *n*-heptane, toluene, and 1,2,4-trimethylbenzene between headspace and a PDMS SPME fiber at 100°C and 130°C.

Then, three model headspace systems containing different compositions of vaporized *n*-heptane, toluene, and 1,2,4-trimethylbenzene were prepared. Chemical analysis of these model headspace systems was conducted via a PDMS SPME sampling method at 100°C and 130°C. The true relative compositions of these vaporized compounds in three model headspace systems were estimated using compositions from a PDMS SPME sampling method and the relative partition coefficients from inverse gas chromatography. Estimated relative compositions were compared to true compositions which were obtained from direct headspace analysis. Except for the composition of 1,2,4-trimethylbenzene in one model headspace at 100°C, the estimated compositions agree with experimental compositions within a relative error of less than 10%.

As presented in this work, inverse gas chromatography can provide an alternative way for the estimation of partition coefficients of volatile compounds at the solid/headspace interface which are critical for the quantification of the actual headspace compositions. Although only three vaporized components were used to establish model headspace for chemical analysis in this work, there is the ability to expand inverse gas chromatography measurements to more components to estimate partition coefficients at various solid/headspace interfaces. In addition, partition coefficients estimated via inverse gas chromatography measurements need to be validated under different experimental conditions such as the presence of more components with various quantities. The results produced in this work and future studies can be applied to the quantification of target compounds in various forensic samples. To achieve goal, inverse gas chromatographic measurements with columns packed by various materials such as fabric, cardboard, and carpet for more hydrocarbons are currently underway.

#### **HS/SPME, Inverse Gas Chromatography, Partition Coefficient**



### **B144 A Biological Sample and a Gunshot Residue Collection From the Shooter's Hand: Which Comes First?**

*Tugba Ünsal, PhD\**, Üsküdar University Department of Forensic Science, Istanbul 34662, TURKEY; *Aylin Yalçın Saribey, PhD*, Uskudar University, Istanbul, TURKEY; *Kaan Yilancioglu, PhD*, Üsküdar University, Istanbul 34662, TURKEY; *Sevil Atasoy, PhD*, Uskudar Universitesi Adli Bilimler Enstitüsü, Istanbul, TURKEY

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**THIS ABSTRACT WAS NOT PRESENTED.**



### **B145 Determining the Confidence of Fingerprint Examiners at Various Steps in the Analysis, Comparison, Evaluation-Verification (ACE-V) Method**

*Kierstyn L. Evans, BS\*, Anson, TX 79501; Megan Thoen, PhD, Texas Tech University, Lubbock, TX 79409-7406*

**Learning Overview:** After attending this presentation, attendees will be familiar with the limited published information regarding the Analysis, Comparison, Evaluation-Verification (ACE-V method) used for fingerprint examination, contributing to a lack of trust or to hesitancy about the findings of analysts who use this method. This study will provide a better understanding of the decisions being made by fingerprint examiners, and their confidence in those decisions, and will help to further validate the use of the ACE-V method.

**Impact on the Forensic Science Community:** This research will impact the forensic science community by providing a better understanding of the details that make up the complex and unstandardized process of the ACE-V method and its use by fingerprint examiners.

The ACE-V method is the most common procedure used by fingerprint examiners to analyze a fingerprint found at a crime scene (unknown donor) and comparing it with a fingerprint in a database (known/exemplar).<sup>1</sup> One problem with this procedure is that there is not a set list of steps to follow that is consistent among all fingerprint examiners; there is not a clear definition of what each step entails, and few steps are standardized.<sup>2</sup>

A problem with this lack of detail and standardization is that each fingerprint examiner, even those from the same agencies, find different 'points' of comparison to match the fingerprints. Points are the areas of a print identified in the unknown print and are matched to those in the exemplar print. These differences in the identification of critical points show the variability of each examiner. Because of this variation in the determined points of comparison, the use of fingerprints as evidence has become scrutinized.<sup>3</sup>

For these reasons, understanding the confidence of fingerprint examiners, at various points throughout the ACE-V method, will help the support the use of this method in a court of law.

This study has four research questions:

1. What is an examiner's level of confidence at various decision points of the ACE-V method, including after determining how many points for comparison exist within the presented fingerprint?
2. Does this level of confidence at each decision point vary depending on level of training?  
Hypothesis: The more experience an examiner has, the higher their reported confidence level when compared with those examiners with fewer years of practice.
3. Does the number of points of comparison identified vary depending on level of training?
4. Does experience impact the conclusion at each decision point of the ACE-V method?

To answer these questions, an online survey will be used to ask fingerprint examiners questions about fingerprint images that appear before them. Requests for participation in the study will be submitted through a variety of various forensic science and criminalistics organizations. The fingerprint stimuli for this study were created under the guidance of current fingerprint examiners and pretested before final dissemination of the survey. The survey includes questions about participants' occupation, years of experience as a fingerprint examiner, the suitability for comparison of the presented fingerprint, the number of points that are found for comparison, and how confident examiners are in their decisions.

Data collection will be completed by January 2019. Anticipated findings include that those fingerprint examiners with more training and experience in using the ACE-V method will find more points of comparison within fingerprint(s) to justify their decisions (i.e., match, exclusion, inconclusive) and will be more confident in those decisions. These anticipated findings will guide future directions for research involving fingerprint examination and the ACE-V method, including discussion of the methodological and practical implications for generating empirical knowledge in this area.

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#### **Fingerprints, ACE-V, Decision-Making Confidence**



### B146 Canine Detection of Organic and Inorganic Gunshot Residues (GSR) on the Suspected Shooters

Marcello Rendine, DBA\*, Department of Forensic Pathology, Foggia 71100, ITALY; Irene Riezzo, MD, PhD, University of Foggia, Osp D'Avanzo, Foggia 71100, ITALY; Carmela Fiore, MD, Ospedale "G. Tatarella", Cerignola, Foggia 71100, ITALY; Michela Ferrara, MD, Department of Forensic Pathology, Foggia 71122, ITALY; Francesco Sessa, MS, Department of Forensic Pathology, University of FOGGIA, Foggia 71100, ITALY; Gianfranco Guccia, Palermo 90145, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY

**Learning Overview:** After attending this presentation, attendees will have a better understanding of how a well-trained scent-detection dog can be an alternative and rapid tool to facilitate the identification of a suspected shooter.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a variety of benefits to law enforcement regarding the need to improve the performance, reliability, and courtroom defensibility of detection dog teams and their optimized combination with forensic operators, showing their capability in detection and identification of gunshot residue.

The identification and the analysis of gunshot residue are important evidences in the field of forensic science and there is a need for the development of tools more rapid and sensitive than stubs or swabs, to obtain more conclusive results.

This study determined that trained detection dogs alert to organic compounds and inorganic components indicative of gunshot residues presence on the hands and the clothing of shooters and non-shooters, maximizing the finding of the shooter in an efficient, cost and time effective manner.

The identification of the suspected shooter may often be ambiguous during initial processing investigation, and the test may not always be obvious after some days if the suspect has not been apprehended immediately because GSR persistence decreases quickly with time.

Furthermore, in cases of foul play, a shooter may attempt to scrub and clean up gunshot residues from his own hands, from clothing, or from other surfaces.

For this reason, the investigation findings and the subsequent scientific evaluation of a suspected shooter require a relatively short response time to avoid contamination and to preserve any and all possible evidence of the shooting.

This study aimed to investigate the baseline detection limits of GSR-detection dogs and to latent gunshot residues evidence on washed hands, face, and clothing, to potentially generate an alert by a detection canine by a *positive predictive value* (PPV), and to compare the dogs' responses to current presumptive chemical and analytical techniques.

Field trials experiments to determine canine interest in the observed GSR samples were conducted.

The canine detection of GSR scent in low concentration is called "*sensitivity*".

In addition, this study reports the analysis of several potential interference odorant compounds (i.e., hands soap, household laundry detergent, perfume, etc.) at these GSR scent traces in minimum concentration, and the associated percentages of false positive alerts (false PPV).

The canine discrimination of GSR scent traces in minimum concentration is called "*specificity*".

After this training, a series of field trials are performed to test the canine's "*limit of detection*" (LOD) for the GSR, which is the lowest quantity of a substance that the dog can be distinguished from the absence of that substance (blank value). The limit of detection has been determined by performing scent line-ups in which various amounts of GSR have been exposed and the lowest concentration of GSR for which the canine can still alert have been recorded.

The study demonstrates that canines are generally not using the relatively low volatility parent substances, but instead use characteristics VOCs (volatile organic compounds) to accurately locate specimen of GSR, with the ability to distinguish between inorganic and organic residues of gunshot. The results of this study indicate that the well-trained gunshot detection dog is an outstanding tool for suspected shooter investigation, displaying excellent sensitivity (100), specificity (99), and having a positive predictive value (99), negative predictive value (99) as well as accuracy (99).

These recovery rates ranged between 99% and 100% indicate that properly trained GSR dogs can make significant contributions in the finding GSR traces and so in the identification the shooter.

#### Gunshot Residue (GSR), Canine Detection and Identification, Suspected Shooter



### B147 An Evaluation of Novel Headspace Extraction Methods for the Analysis of Ignitable Liquid Residues (ILRs)

Michelle N. Torres, BS\*, Florida International University, Miami, FL 33199; Nicole Valdes, BA, Florida International University, Miami, FL 33199; Jose R. Almirall, PhD, Florida International University, Miami, FL 33199

**Learning Overview:** After attending this presentation, attendees will better understand the performance capabilities of a novel headspace extraction devices for ignitable liquid residues.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting an emerging headspace sampling technique that is faster, more sensitive, and customizable for the analysis of fire debris.

Forensic analysts have an array of techniques to choose from when assessing fire debris for the presence of ignitable liquid residues (ILRs). In some instances, a technique like solvent extraction is used but is generally not favored due to solvent waste and destruction of the evidence. The techniques that see the most use in modern laboratories are passive headspace-based; adsorption on to an activated charcoal strip (ACS) or a solid-phase microextraction (SPME) fiber are the most popular choices.<sup>1</sup> These are non-destructive and do not require prior sample preparation. However, both techniques have notable disadvantages. Sampling with a charcoal strip necessitates the use of a toxic solvent such as carbon disulfide to desorb compounds and can be subject to the displacement of smaller molecules. The equilibrium-based SPME technique also suffers from this because it has a very limited extraction surface area. The overall sampling time for both techniques is also relatively long. Charcoal strips are normally exposed to the headspace between 2 -16 hours during extraction and SPME fibers require at least an hour of conditioning after every use to prevent carryover.<sup>2</sup>

In this study, the effectiveness of ACS and SPME for the analysis of ILRs is evaluated in comparison to capillary microextraction of volatiles (CMV). The CMV is a dynamic headspace sampling device which consists of a dual open-ended capillary tube filled with glass microfiber strips that are coated in a PDMS-incorporated sol-gel polymer. The sol-gel formulation has been modified with specific precursors to provide high adsorption affinity for a range of compounds and an increase in surface area over SPME of 5,000 times. Sampling is achieved in as little as 10 minutes, and the construction of the device allows the CMV to be thermally desorbed by direct introduction into a GC/MS inlet. Previous applications of the CMV include the headspace sampling of smokeless powders, marijuana plant material, and BTEX vapors.<sup>3-5</sup>

The three techniques are evaluated based on several points of comparison, including generated chromatographic patterns, the total length of time needed for equilibration and extraction, and percent mass recovered. Quantitative analysis is based on a representative '10-mix' of standard ILR compounds ranging from toluene to eicosane. The sampling system for the CMV has been previously reported and has been optimized for the extraction of ILRs.<sup>5</sup> The sampling conditions for ACS and SPME are in accordance with recommendations from their respective ASTM methods. Simulated charred debris samples are examined, including wood, cotton, and drywall substrates that have been doused with either gasoline or diesel in various weathered states. CMVs constructed with multiple functionalized sol-gel strips are also evaluated as an alternative to multi-phase SPME fibers.

The data presented here will demonstrate the potential of the CMV as an alternative sampling method for ILR extraction. These initial demonstrations of high sensitivity, fast analysis time, and general ease of use will hopefully encourage practicing fire debris analysts to explore the viability of the CMV in a routine laboratory setting.

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#### Fire Debris Analysis, Headspace Sampling, Ignitable Liquid Residues



### **B148 Snapshotting Individuals From Nose Shape Genetics—A Novel Tool of DNA Witnesses**

*Zeenat Salahuddin, PhD\**, University of Health Sciences, State College, PA 16801; *Tomás B. González*, Pennsylvania State University, University Park, PA 16803; *Julie White, MA*, Pennsylvania State University, University Park, PA 16803; *Peter Claes*, KU Leuven, Leuven, Vlaams Brabant, BELGIUM; *Susan Walsh, PhD*, Indiana University Purdue University Indianapolis, Indianapolis, IN 46202; *Mark D. Shriver, PhD*, Pennsylvania State University, University Park, PA 16802; *Allah Rakha, PhD*, University of Health Sciences, Lahore 5400, PAKISTAN

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**THIS ABSTRACT WAS NOT PRESENTED.**





### **B149 Comparison of Methods for Developing Submerged Fingerprints**

*Irene M. Chiang, BS\*, University of Alabama at Birmingham, Birmingham, AL 35233-1303; Elizabeth A. Gardner, PhD, University of Alabama at Birmingham, Birmingham, AL 35294-4562*

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**Learning Overview:** Learning Objective: After attending this presentation, attendees will learn the factors affecting the method selected for developing fingerprints that have been immersed in water.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comparison of methods for developing fingerprints on non-porous materials and immersed in water. Variables discussed include print deposition before or after immersion, glass or aluminum substrate, and length of immersion.

Fingerprints found at crime scenes are valuable evidence for identification. The composition of latent fingerprints includes both eccrine and sebaceous secretions, which are targeted by physical or chemical methods to visualize the prints. The method for developing latent fingerprints is selected based on the composition of the fingerprint residues, the surface, and the environment.

Evidence exposed to wet environment may be neglected, while previous studies have proven that fingerprints on non-porous substrates and immersed in water for up to fifteen days can be developed successfully by small particle reagent (SPR). Specifically, SPR was found to performed well on plastic and aluminum foils. Another well-known method for developing prints on non-porous surfaces is cyanoacrylate fuming. Both SPR and cyanoacrylate fuming methods have shown promise for developing identifiable prints on surfaces recovered from wet environment. A new method, Oil Red O has recently been proposed for the development of fingerprints on wet surfaces. Oil Red O is a lipophilic dye that binds to the sebaceous residues in a fingerprint and stains the print pink.

In this project, Oil Red O was compared to SPR (Sirchie SPR100) and cyanoacrylate fuming on fingerprints deposited on glass slides and aluminum foils. Since fingerprints at crime scenes can be left on wet surfaces or made under water, the experimental prints were deposited both before and after the substrates were submerged. The substrates were immersed in distilled water for 1, 3, or 7 days. After development, the minutiae are marked by using AFIX software. The quality of the developed prints is reported as the percentage of minutiae present in the experimental print compared to the number in the reference print.

Initial results indicate that SPR produces the best results for fingerprints submerged for seven and three days, though the quality is lower than that of the control print. Moreover, the performance of the SPR is not influenced by whether the prints were deposited before or after submersion. The prints submerged for seven days are visible after the development of cyanoacrylate fuming and Oil Red O solution, but the prints fail to be lifted and preserved for identification. However, the prints made under water which were immersed for three days can be developed by cyanoacrylate fuming and Oil Red O solution. To date, the less time samples are in contact with water, the more effective the methods are for developing the print.

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#### **Wet Fingerprints, Small Particles Reagent, Oil Red O**



### B150 Pyrolysis Direct Analysis in Real-Time Mass Spectrometry (DART®-MS) Analysis of Ignitable Liquids With Chemometrics

Isabella C. Barnett, BA\*, Isabella Barnett, Clarksville, TN 37043; Mengliang Zhang, PhD\*, Middle Tennessee State University, Murfreesboro, TN 37132

**Learning Overview:** The goals of this presentation are to: (1) distinguish among different classes of petroleum-based products, and (2) classify gasoline samples based on their brands by using Direct Analysis in Real-Time Mass Spectrometry (DART®-MS) technique and chemometrics. This research concentrates on analyzing the effects and reliability of the IonRocket system, a pyrolysis device to couple with DART®-MS to assist in analysis of samples on substrates.

**Impact on the Forensic Science Community:** After attending this presentation, attendees will better understand how DART®-MS and the IonRocket system can be an alternative method for petroleum product identification in forensic arson investigations. This research is a pioneer for gradient heating-controlled pyrolysis DART®-MS, which will hold great potential across a broad range of topics in the analytical chemistry area and within forensic arson investigations.

The evidential link between a suspect and a crime scene could be established based on the identification of fuels or the source of them. Ambient mass spectrometry methods such as direct analysis in real time mass spectrometry (DART®-MS) can be an alternative method for petroleum product identification which enables the direct sample analysis without chromatographic separation steps.<sup>1</sup> DART®-MS method requires limited sample preparation and is applicable to the analysis of both volatile and nonvolatile compounds with excellent analytical sensitivity.<sup>2</sup> The objectives of this research were: (1) to distinguish among different classes of petroleum-based products, and (2) to classify gasoline samples based on their brands by using DART®-MS technique and chemometrics. This research concentrates on analyzing the effects and reliability of the IonRocket system, a pyrolysis device to couple with DART®-MS to assist in analysis of samples on substrates.

The pyrolysis device, IonRocket, was coupled with DART®-MS to assist the analysis. IonRocket system is an ideal unit for the analysis of polymers with which the samples can be heated up to 600°C with accurate temperature gradient control for DART®-MS analysis. The temperature was increased at the rate of 100°C per minute, so data with three dimensions consisting of  $m/z$ , time (=temperature), and intensity was generated which further enhanced the discriminating power comparing with sole DART®-MS analysis when chemometrics was applied. The pyrolysis DART®-MS system was used for the classification of various petroleum distillates such as gasoline, kerosene, paint remover, lighter fuel, and so on, but also for the differentiation of gasoline from different gas station chains based on the profiles of fuel additives. Other characteristics were analyzed over the course of this research, including time profiles, detection limits, and presence of the ignitable liquids on multiple substrates.

Chemometrics is critical for both data preprocessing and statistical analysis in this case. Statistical and chemometric methods have been widely used to increase the confidence in the association or discrimination and build more definitive links between samples in forensic research.<sup>3-4</sup> In this study, different data preprocessing techniques such as binning, normalization, scaling, and data transformation were tested and both unsupervised and supervised statistic models such as principal component analysis (PCA), partial least square discriminant analysis (PLS-DA), and soft independent modeling of class analogy (SMICA) were established and validated for the differentiation of gasoline with different brands.

This presentation will describe the use of pyrolysis DART®-MS technique and chemometric models for the classification of ignitable liquids and characteristic features to identify each ignitable liquid. The impact of different substrates and weathering will be further discussed.

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#### DART®-MS, Ignitable Liquids, Chemometrics



### **B151 Mosquitoes: A Potential Source for Human Identity**

*Shayna L. Gray, BS\*, Pennsylvania State University, University Park, PA 16802; Scott Lindner, PhD, Pennsylvania State University, University Park, PA 16802; Reena Roy, PhD, Pennsylvania State University, University Park, PA 16802*

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**Learning Overview:** After attending this presentation, attendees will learn how substrates other than those normally found in crime scenes can be used for human identity. Evidence such as insects can be useful for aiding in an investigation of a crime.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how the project utilizes a combination of direct amplification and massively parallel sequencing of DNA from the midgut of mosquitoes to aid the human identification process.

A combination of short tandem repeat sequence and single nucleotide polymorphism analysis using capillary electrophoresis and massively parallel sequencing technology allows for identification of the donor of the blood on which the mosquito has fed.

Evidence such as blood or saliva containing deoxyribonucleic acid (DNA) can be helpful in linking an individual to a crime scene. Common substrates from which DNA can be obtained at a crime scene include different types of fabric, objects used to drink or eat, cigarette butts, leaves, wood, grass, tools, and similar objects. Traditionally, DNA is obtained from body fluids deposited on substrates such as these. One of the more unusual sources that can be used for generation of DNA profiles are insects such as mosquitoes or flies. In addition to their role as infectious vectors for malaria and other diseases, mosquitoes are also useful from a forensic perspective. These insects are not generally included as potential sources of human DNA even though female mosquitoes feed on human blood and store the blood in their midgut for a period of time after feeding. This stored blood contains nucleated white blood cells that house nuclear DNA, which can potentially be used to identify the individual from which the blood meal is taken. Additionally, mosquitoes maintain a ubiquitous presence in the environment, especially in the summer or in geographical areas with hot climates. Therefore, it would not be unusual for them to be present at crime scenes. In fact, mosquitoes have been previously documented and collected at crime scenes around the world.

The primary goal of this research was to obtain forensic short tandem repeat (STR) DNA profiles as well as detect single nucleotide polymorphisms (SNPs) from the blood in the abdomen of mosquitos. Direct amplification using the PowerPlex® Fusion 6C System from Promega Corporation and the Investigator® 24plex GO! Kit from Qiagen was used to detect STR profiles. SNP analysis was performed using the Precision ID Identity Panel on the S5™ system from ThermoFisher. Mosquitoes were euthanized by freezing or by submerging the mosquitoes in ethanol at different time intervals after they were fed on human blood meals. These blood meals included both human blood, from single sources as well as mixtures. Another goal of this research was to determine the time when DNA from the blood starts to degrade within the abdomen of these insects after feeding.

It was concluded that DNA contained in the abdomen of the mosquito can be used to determine the donors' identity using both the STR and SNP technologies. The results indicated that profiles obtained from the blood in the mosquitoes were consistent with profiles obtained from reference blood of the same source. DNA from mosquitoes euthanized at different time intervals was assessed for degradation and it was determined that DNA was completely degraded when blood remained in the abdomens of these mosquitoes for 72 hours.

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#### **DNA Analysis, Massively Parallel Sequencing, Mosquitoes**



### **B152 Small Unmanned Aerial System (sUAS) Attribution Phenomenology Via Plant/Fungal and Human DNA Sequencing**

*Josh Dettman\**, Lexington, MA; *Tara Boettcher, BS*, MIT Lincoln Laboratory, Lexington, MA 02421; *Jim Comolli, PhD*, MIT Lincoln Laboratory, Lexington, MA 02421; *Martha Petrovick, PhD*, MIT Lincoln Laboratory, Lexington, MA 02420

**Learning Overview:** After attending this presentation, attendees will understand: (1) how signatures like trace/touch human DNA (hDNA) mixtures and plant/fungal environmental DNA (eDNA) may enable attribution of small unmanned aerial systems (sUAS) to people and places, (2) where successful signature collections occurred on sUAS as part of operationally-relevant experiments, and (3) how similar forensic phenomenology experiments conducted under realistic conditions can inform improvements in the practice of forensic science.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting initial research findings related to exploitation of signatures to identify builders, pilots, or others who have handled the sUAS (human DNA sequencing) and testing locations (eDNA sequencing). Use of sUAS by the general public is continuing to increase, and law enforcement should expect an increasing number and variety of encounters with sUAS platforms and users with both benign and nefarious intentions.

Capabilities to attribute sUAS to builders/pilots and production/testing locations are important to deter and prevent their misuse. Attribution is enabled, in part, through understanding the production and operational networks that utilize these devices. To provide a supplement to exploitation of flight paths and other sUAS electronic data, attribution signature collection and analysis in this study was focused on.

Analysis of trace/touch human DNA mixture using single nucleotide polymorphism (SNP) sequencing to link unknown biometric identities (UBIs) of builders/pilots across different sUAS platforms (and by comparing profiles to those in a reference database where possible);

Analysis of plant and fungal eDNA found in dust collected from sUAS with metabarcoding and DNA sequencing to associate traces of species on the device with those found in previous testing locations.

Both naturally deposited and artificially added positive control DNA signatures were collected from nine different sUAS types under three potential law enforcement sUAS encounter scenarios: (1) pre-flight (prior to sUAS use), (2) post-flight (after successful flight and landing), and (3) post-crash (after unsuccessful landing). In all, 147 samples were collected at an initial test effort in Maryland, and 222 samples were collected during the main test event in Nevada.

The human DNA results from this data set indicate that recovery of DNA profiles from swabs of sUAS components post-flight (88% of samples yielded at least one DNA profile) and post-crash (92% of samples collected) is possible. Additional tests are needed to inform final recommendations regarding priority collection locations, but samples collected from commonly touched areas like circuit boards, internal component covers that require pressing down to secure, hand-launch points, and tape samples yielded sufficient quantity and quality of DNA for UBI detection and association in this study. Because the DNA naturally deposited on these sUAS was low-concentration touch samples with mixtures of contributors, current short tandem repeat (STR) analysis will be challenged to provide DNA profiles.

The initial results also indicate that there was sufficient recoverable eDNA from sUAS, both from post-flight and post-crash sampling, to assess the ability identify previous testing locations of the sUAS. DNA sequences corresponding to more than 10 different fungal and plant species were recovered from 94% of sUAS post-flight and post-crash samples. Recovery of eDNA and the number of identified fungal/plant species was similarly high across the samples from different exterior sites on the sUAS surfaces, so no preferred sampling site was obvious. An average of 17% of the identified species in samples from sUAS originally operated in Maryland, then in Nevada, were found in reference samples from the Maryland testing location. This demonstrated that plant and fungal species characteristic of previous locations could be used to identify previous testing locations of the sUAS. Results indicated that additional sampling from the sUAS and reference site to improve species coverage would increase the likelihood of definitive, statistically significant geographic attribution.

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#### **Small Unmanned Aerial System, Phenomenology, DNA Sequencing**



### **B153 Internal Validation of the “DogFiler” Short Tandem Repeat (STR) Amplification Assay for the Analysis of Canine DNA Evidence**

*Sam Kwiatkowski, PhD\*, Harris County Institute of Forensic Sciences, Houston, TX 77030; Michael A. Donley, MS, Houston, TX 77021; Katherine Welch, MS, Harris County Institute of Forensic Sciences, Houston, TX 77054; Roger Kahn, PhD, Harris County Institute of Forensic Sciences, Houston, TX 77054*

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**Learning Overview:** The goal of this presentation is to demonstrate preliminary validations for the “DogFiler” canine STR amplification assay. The long-term goals are to address the challenges of present canine STR technology and to model a strategy for canine STR analysis for the forensic science community. DogFiler was selected among other canine STR assays due to the accessibility to an allelic ladder and an allele frequency database.

**Impact on the Forensic Science Community:** This presentation will impact the forensic scientific community by demonstrating competence and enabling performance of canine STR analysis by public crime laboratories.

Dogs may be victims, perpetrators, or witnesses to crime, leaving traces of DNA evidence in various types, such as blood and hair, which may be analyzed using canine-specific STR assays. Results may link individual dogs to a crime scene or person of interest. Canine DNA analysis has been ruled as admissible evidence in court for numerous cases involving abuse, burglary, assault, and homicide.

Public crime laboratories have been slow to incorporate canine DNA analysis as a regular internal function. This is due in part to the lack of required tools, for example, allelic ladders and allele frequency databases, lack of participation by public crime laboratories in validations and casework application, and limitations on the robustness of current canine STR assays. Improvements are needed to promote the advantages of canine STR analysis as tool for human criminal investigations, and to facilitate wider application in public crime laboratories.

DNA was extracted from canine sources, quantified, amplified with DogFiler, and analyzed by capillary electrophoresis. Sizing was accomplished by comparing resultant peaks to an allelic ladder. Internal validation studies included determining sensitivity, evaluating stutter, resolving two-contributor mixtures, and analyzing mock casework samples. Mock casework samples included individual shed hairs comprising bulb tissue. Bundles of shed hair were analyzed as potential evidentiary samples. Bundles of hair collected directly from self-grooming locations were analyzed as potential alternate reference samples.

The sensitivity study showed that canine profiles could be generated from as little as 0.2ng template. DogFiler was used to resolve two-contributor mixtures across varying mixture ratios and input amounts. Full profiles were generated from shed hair bundles and hair bundles collected from self-grooming locations. Self-groomed hair bundles sometimes exhibited significantly greater DNA concentration likely due to the presence of saliva due to grooming. The use of an allelic ladder during profile interpretation promoted standardization in allele sizing. Future goals include applying DogFiler to casework investigations at Harris County Institute of Forensic Sciences (HCIFS) and promoting the application of canine STR analysis in public crime laboratories across the United States.

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#### **Canine, Short Tandem Repeat, DogFiler**



### B154 The Utility of the Human Hair Microbiome in the Forensic Analysis of Human Hairs

Tyler Kennedy, BS\*, Fredericksburg, VA 22406; Baneshwar Singh, PhD\*, Virginia Commonwealth University, Richmond, VA 23284

**Learning Overview:** After attending this presentation, attendees will gain a better understanding on bacteria associated with human hair and its temporal stability.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a foundation about the human hair microbiome and its potential use in casework.

Hair is among the most commonly found pieces of evidence at a crime scene, yet it doesn't contain much probative value unless the root is present. Previous research has explored the hair microbiome to unlock evidentiary hair's full probative potential, but they used small sample sizes. The main goals of this study were to identify the core bacterial taxa associated in human pubic and scalp hairs in both sexes and examine the temporal stability of the hair microbiome at room temperature. To accomplish these goals, pubic hair (n=58; 33 female and 25 male), and scalp hair (n=65; 40 female and 25 male) sample were collected and stored at 25°C for zero week (Baseline), six weeks, and twelve weeks in an incubator. DNA extraction was performed within a day after collection for baseline samples and after six and twelve weeks for other samples. 16S ribosomal DNA (16S rDNA) high-throughput sequencing was performed on all samples using dual-index strategy on Illumina MiSeq FGx sequencing platform. Data analysis was performed in mothur and in SPSS. Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes constitute almost 97% of all bacteria associated with human hairs. In both sexes, bacterial diversity of pubic hair was much higher than bacterial diversity of scalp hair. Significant difference in bacterial structure was observed between pubic hair and scalp hair. This difference was mainly because of high relative abundance of Proteobacteria in scalp hair. Bacterial structure was also significantly different between male and female pubic hairs. This difference was mainly because of high relative abundance of *Lactobacillus*, *Prevotella*, and *Gardnerella* in female pubic hair. Although, bacterial structure associated with female scalp hair was significantly different than bacterial structure associated with male scalp hair in Chi square test, the same was not true in analysis of molecular variance (AMOVA). In both pubic and scalp hair, bacterial diversity decreased with increase in storage time at room temperature. Bacterial structure associated with baseline samples were significantly different than bacterial structure associated with twelve weeks samples, and this difference was mainly because of increase in relative abundance of Betaproteobacteria with storage time. In conclusion, the study provides evidence that time, origin on body, and sex all play a role in the hair microbiome and understanding these variables provides a foundation for using hair in future forensic analysis of hair.

#### Human Hair, Bacteria, 16s rDNA



### **B155 The Characterization of New Chloroplast Markers to Determine the Biogeographical Origin and Crop Type of *Cannabis Sativa* Samples**

Madeline G. Roman, BS\*, Sam Houston State University, Huntsville, TX 77340; Rachel M. Houston, PhD, Sam Houston State University, Huntsville, TX 77340; Bobby Larue, Jr., PhD, Sam Houston State University, Huntsville, TX 77340; David A. Gangitano, PhD, Sam Houston State University, Huntsville, TX 77340

**Learning Overview:** After attending this presentation, attendees will understand a novel research project which seeks to: (1) provide a comprehensive genetic tool to identify *cannabis sativa*, (2) distinguish between hemp and marijuana samples, and (3) determine the biogeographical origin of samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing investigative leads for law enforcement and providing evidence for linking cases, distributors, growers, or individual samples.

Marijuana (*Cannabis sativa*) is the most commonly used illicit drug in the United States, and despite its schedule I classification by the federal government, 29 states and the District of Columbia have legalized its use for medicinal or recreational purposes. This state-specific legalization has created a new problem for law enforcement, which must now investigate the diversion of legally-obtained *Cannabis* to states where it remains illegal. In addition, illegal trafficking of the drug at the border with Mexico remains a substantial issue for law enforcement agencies.

*C. sativa* crops can be broadly classified as marijuana (a drug containing the psychoactive chemical delta-9-tetrahydrocannabinol, or THC) or hemp (the non-drug form of the plant, cultivated for oil and fiber), and differentiation between crop types is important for determining whether the crop is a controlled substance. In addition, investigation of trafficking routes into and within the United States requires genetic association of samples from different cases/seizures, and chloroplast DNA (cpDNA) markers may help to provide investigational leads or link cases with common growers or distributors.

This project seeks to exploit polymorphisms in *C. sativa* cpDNA to provide the forensic community with a comprehensive analytical tool that will allow genetic determination of biogeographic origin, discrimination between drug-type *Cannabis* and fiber-type *Cannabis* samples (marijuana vs. hemp), authentication of medical marijuana samples, and association between cases for *C. sativa* samples. By comparing published cpDNA sequences, 58 polymorphisms were identified, including several “hotspot” regions. This presentation will discuss the process of finding these polymorphisms and designing and optimizing custom Sanger sequencing, fragment, and SNaPshot™ analyses to genotype the chosen “hotspot” regions. Haplotype results from hemp and marijuana samples from four countries (United States, Canada, Mexico, and Chile) will be discussed, as well as phylogenetic analyses evaluating the utility of the chosen polymorphisms in grouping samples from different geographical origins and crop types. This is an ongoing project, and future research will focus on expanding the number of polymorphic markers, designing and validating a method to sequence the informative markers on a massively parallel sequencing platform, and building a database of cpDNA haplotypes for *C. sativa* samples.

**Forensic Plant Science, Chloroplast DNA Barcoding, *Cannabis sativa***



### **B156 Massively Parallel Sequencing (MPS) of 12 Autosomal Short Tandem Repeats (STRs) in *Cannabis Sativa***

Rachel M. Houston, PhD\*, Sam Houston State University, Huntsville, TX 77340; Carrie Mayes, BS, Sam Houston State University, Huntsville, TX 77340; Jonathan King, MS, Fort Worth, TX 76107; Sheree R. Hughes-Stamm, PhD, Sam Houston State University, Huntsville, TX 77340; David A. Gangitano, PhD, Sam Houston State University, Huntsville, TX 77340

**Learning Overview:** After attending this presentation, attendees will understand the basic principles behind applying Massively Parallel Sequencing (MPS) techniques to sequencing autosomal STR markers in *Cannabis sativa*.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the applicability of an autosomal MPS panel that could not only assist law enforcement agencies in verifying legal marijuana products, but also aid in the linkage of illegal cases. This method could also serve as an additional tool to previously established marijuana profiling programs used in federal agencies such as U.S. Customs and Border Protection (CBP) and the Drug Enforcement Administration (DEA). Importantly, the methods presented could also be applied to integrate any custom PCR multiplex into a MPS pipeline.

Massively parallel sequencing (MPS) is an emerging technology in the field of forensic genetics that provides distinct advantages compared to capillary electrophoresis CE. While CE offers a reliable and robust technique, it has disadvantages such as limited multiplexing capability with a maximum of 25 to 30 loci configurable across five dye channels. In addition, MPS has the potential to provide deeper interrogation of sequence-based polymorphisms, which in turn allows for a greater power of discrimination compared to size-based STR genotyping by CE. Currently, no targeted MPS workflows have been used for *C. sativa*. Targeted sequencing is necessary for forensic comparisons and custom MPS panels can be designed by manufacturers. However, *C. sativa* is not currently a supported species for commercial MPS panels and targeted sequencing panels need to be designed in-house.

This study offers a proof of concept that MPS technologies can be applied to genotype autosomal short tandem repeats (STRs) in *Cannabis sativa*. A custom panel for MPS was designed to interrogate 12 cannabis-specific STR loci by sequence rather than size. A simple workflow was implemented to integrate the custom PCR multiplex into a workflow compatible with the Ion Plus Fragment Library Kit, Ion™ Chef, and Ion™ S5 System. For data sorting and sequence analysis, a custom configuration file was designed for STRait Razor v3 to parse and extract STR sequence data. This study represents a preliminary investigation of sequence variation for 12 autosomal STR loci in 16 cannabis samples from three different countries.

Results demonstrated that MPS can be used to genotype autosomal STRs in *C. sativa* and revealed intra-repeat variation in eight loci where the nominal or size-based allele was identical, but variances were also discovered in the sequence of the flanking region. MPS performance including read depth, heterozygote balance, noise, and CE concordance was assessed. Complete concordance was observed between the two methods when comparing the length-based alleles extracted by STRait Razor and the allele number observed by CE. Although only a small number of cannabis samples were evaluated, this study demonstrates that more informative STR data can be obtained via MPS. In addition, this study reveals a workflow that can be used to integrate any custom PCR multiplex into a MPS workflow.

Overall, this research investigates the sequence variation of 12 autosomal STR loci in 16 *C. sativa* samples and provides a proof of concept that MPS can be applied to genotype *C. sativa* samples.

#### **Forensic Botany, *Cannabis sativa*, Massively Parallel Sequencing**





### **B157 The Development of a Method to Extract Opium Poppy (*Papaver Somniferum L.*) DNA From Heroin**

Michael Marciano, MS\*, Forensic and National Security Sciences Institute, Syracuse, NY 13244-4100; Sini Panicker, Dulles, VA 20166; Garrett Liddil, Manlius, NY 13104-9661; Danielle Lindgren, MS, Austin, TX 78702; Kevin S. Sweder, PhD, Forensic and National Security Sciences Institute, Syracuse, NY 13244

**Learning Overview:** After attending this presentation attendees will be aware of the ability to purify poppy DNA from heroin samples. It will also educate attendees on alternative means to extract DNA from exceedingly low level or difficult samples including techniques to maximize yield and combat PCR inhibitors.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the ability to obtain poppy DNA from heroin samples, opening a door to new types of analyses that could involve DNA-based opioid identification and drug geosourcing. It will also stimulate conversation regarding alternative methods of DNA extraction including using methods that are not commonly used in forensic science.

This study is the first to report the successful development of a method to extract opium poppy (*Papaver somniferum L.*) DNA from heroin samples. Determining of the source of an unknown heroin sample (forensic geosourcing) is vital to informing domestic and foreign policy related to counter narcoterrorism. Current profiling methods focus on identifying process-related chemical impurities found in heroin samples. Changes to the geographically distinct processing methods may lead to difficulties in classifying and attributing heroin samples to a region/country. This study focuses on methods to optimize the DNA extraction and amplification of samples with low levels of degraded DNA and inhibiting compounds such as heroin. The authors compared modified commercial-off-the-shelf extraction methods such as the Qiagen Plant, Stool and the Promega Maxwell-16 RNA-LEV tissue kits for the ability to extract opium poppy DNA from latex, raw and cooked opium, white and brown powder heroin and black tar heroin. Opium poppy DNA was successfully detected in all poppy-derived samples, including heroin. The modified Qiagen stool method with post-extraction purification and a two-stage, dual DNA polymerase amplification procedure resulted in the highest DNA yield and minimized inhibition. This study describes the initial phase in establishing a DNA-based signature method to characterize heroin.<sup>1</sup>

Heroin has been identified as the single greatest drug threat in the United States.<sup>2</sup> The number of individuals using heroin approached one million by the end of 2014, an increase from previous years and, in 2016, has led to an estimated 15,469 deaths, more than a 600% increase since 2002.<sup>3-5</sup> The 2016 National Forensic Laboratory Information System report identified heroin as the 4<sup>th</sup> most common drug case (11.2%). The number of heroin cases submitted to forensic laboratories has recent spiked, with over a two-fold increase in 2015. This is in contrast to trends observed with Cocaine, MDMA, and Cannabis.<sup>6</sup> The heroin epidemic extends beyond domestic health issues. The 2016 United Nations Office on Drugs and Crime report estimated that 1,600 organized criminal organizations in the European Union are involved in drug trafficking making it the predominate illegal activity among human trafficking, fraud, and smuggling.<sup>6</sup> The link between heroin and national security is evident, affecting the health and well-being of Americans, as well as their security. Thus, understanding the flow of drugs from source to end-point becomes critical on federal, state and local levels.

The United States has prioritized the monitoring and diversion control of heroin and other opiates through the assembly of task forces and programs on all governmental levels. These include: the High Intensity Drug Trafficking Areas program, Organized Drug Enforcement Task Force, the Heroin Signature Program, and the Heroin Domestic Monitoring Program. These programs have led to significant gains in the understanding of international and domestic drug trafficking patterns and have aided in the control of the heroin epidemic. The development of a method to isolate poppy DNA from heroin will enable a means of heroin identification that augments the current suite of assays used by the Heroin Signature program and ultimately lead to further insights into drug trade and related insurgent activities.

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#### **Heroin, DNA Extraction, Opium**



### B158 The Use of Optical Profilometry to Characterize Fabric Impressions in Vehicle Surfaces

*Abigail J. Bender, BS\*, Pennsylvania University, University Park, PA 16802; Jessica E. Hovingh\*, State College, PA 16803; Ralph R. Ristenbatt, III, MS, Pennsylvania State University, University Park, PA 16802*

**Learning Overview:** After attending this presentation, attendees will better understand the connection between visualization, impact force, and depth measurements of three-dimensional fabric impressions in vehicle surfaces.

**Impact on the Forensic Science Community:** This study will impact the forensic science community by providing criminalists with an additional investigative and reconstructive aid for vehicle-pedestrian collisions.

Vehicle-pedestrian collisions often result in two-way transfers, in which physical evidence is transferred from the vehicle to the victim and vice versa. Transfers often include biological matter, textile material, and fragments of various automotive components, such as paint, glass, and other synthetic materials. Another form of evidence that may be present is impression evidence from the vehicle's tires, either at the scene or on the victim; additionally, fabric impressions may be formed in the vehicle's paint or other components. Upon impact with sufficient force, the structure of the fabric from the victim's clothing can be impressed in the automotive finish, resulting in a three-dimensional (3D) imprint. While the primary focus of forensic investigations of vehicle-pedestrian incidents heavily relies on the association of transfer evidence between victim and suspect vehicle, 3D fabric impressions could provide criminalists with an additional investigative tool. Several factors, including impact force, composition and construction of fabric, and the physicochemical composition of the automotive finish, likely play an important role in the formation of fabric impressions in vehicle surfaces. The goal of this project is to further investigate these factors and better characterize the formation of fabric impressions.

Impressions were generated using a large-scale pendulum impact device located at the Pennsylvania State University College of Engineering Civil Infrastructure Testing and Evaluation Laboratory (CITEL). The pendulum was equipped with a 6-foot long quick release arm mounted a in 9-foot frame; a weld cap attached to the arm served as a simulated knee. The simulated knee was covered with a piece of denim fabric from Levi's 550™ jeans over a layer of ½-inch thick foam. Samples that were impacted were collected from known vehicle hoods, trunks, doors, and body panels. These vehicle samples were cut to approximately 5 inches by 5 inches and secured into a sample holder with a ¼-inch thick steel backing plate. The pendulum arm height was adjusted to alter impact height thereby varying impact force. While the textile material and vehicle test substrate were held constant, impact height was adjusted to produce fabric impressions of varying visibility and depth.

This study utilized a Zygo® Nexview™ 3D Optical Profilometer, a noncontact and nondestructive instrument. The optical profiler was used to conduct vertical and lateral scans of the impacted surfaces, producing three-dimensional surface topography images of the substrate. This permitted visualization of fabric impressions on a micro-scale and the ability to quantitatively measure impression depth. Preliminary scans of fabric impressions using the optical profilometer showed an increase in the visibility of fabric impressions as impact height increased. If better understood and characterized, fabric impression evidence could serve as an additional aid in the investigation of vehicle-pedestrian collisions.

#### **Vehicle-Pedestrian Collision, Fabric Impression, Optical Profilometry**



### **B159 On the Development of Score Rules for the Pairwise Sample Comparison of Particle Micromorphometry of Aluminum (Al) Powders**

*Danica Ommen, PhD\**, Iowa State University, Department of Statistics, Ames, IA 50011; *JenaMarie Baldaino, MS*, Quantico, VA 22135; *Cami Fuglsby, MS*, Department of Mathematics and Statistics, Brookings, SD 57007; *Christopher P. Saunders, PhD\**, Brookings, SD 57006; *Jack Hietpas, PhD*, Pennsylvania State University, University Park, PA 16802; *JoAnn Buscaglia, PhD\**, FBI Laboratory, CFSRU, Quantico, VA 22135

**Learning Overview:** After attending this presentation, attendees will understand various strategies for the interpretation and presentation of the forensic evidence associated with the comparison of samples of aluminum powders with an automated particle micromorphometry and the associated statistical analyses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the application of aluminum particle micromorphometry as a quantitative method for the characterization and comparison of explosive evidence, which may also provide valuable lead identification for forensic investigations.

Starting materials for an Improvised Explosive Device (IED) are readily obtainable from local commercial sources. Aluminum (Al) powder, a common metallic fuel, has a wide variety of legitimate uses and is widely available without significant regulatory constraints.<sup>1</sup> Al powders can be obtained from industrial manufacturers or can be produced inexpensively using basic instructional manuals and videos. Due to the on-line sharing of instructional manuals and published books on how to construct IEDs, bomb makers are now informed on the easily accessible household materials that can be used to make explosive chemical mixtures.<sup>2</sup>

Previous results using scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDS) showed morphology and surface characteristics can differentiate some methods of Al powder manufacturing (i.e., industrial vs. homemade). Particle micromorphometry may be used as a complementary method to gain additional information to differentiate Al powder sources. This presentation builds on our past results and is focused on the development of efficient scoring rules for measuring the similarity (and dissimilarity) between two samples/sources (sets) of Al powder based on the two distributions of particles.

Al powder samples were obtained from legitimate industrial manufacturers, various amateur production methods, and seized IEDs. The amateur methods were replicated in-house to produce Al powder from easily available sources, including different brands of each of Al foil, metallic spray paints, Al ingots melted from Al cans that had been filed or lathed, pyrotechnics, and catalyst packets from two brands of binary exploding targets. To prepare microscope slides for imaging, a subsample containing ~1,000µg from bulk Al powder was placed into a microtube containing Permount® mounting medium. The solution was mixed until evenly dispersed, then an aliquot of the subsample was placed dropwise onto a microscope slide and a coverslip added. A subset of Al powder samples prepared using seven (7) subsamples and three (3) aliquots for each subsample will be considered in this presentation.

Transmitted light microscope images of the Al samples (with approximately 4,200 fields of view/sample) were acquired using an automated stage and automated Z-focus. Dimensional analysis was calibrated using a National Institute of Standards and Technology (NIST) -traceable stage micrometer; polystyrene spheres of 100µm, 50µm, and 10µm were used as secondary standards to assess linear calibration. Images were batch-processed using commercial image analysis software and customized code. Each image was converted to a binary image to enhance edge detection and the particles were counted and measured. Seventeen (17) parameters were measured for each particle within the image field of view, including various size and shape parameters. The large multidimensional datasets with between 90,000 and 500,000 particles per sample were analyzed using an open source statistical package.

The datasets are too large and complex to analyze with standard statistical methods in an efficient and scalable manner. Current work has focused on developing methods that will compare two sets of particles and summarize the difference in the distribution of particles with each particle characterized by 17 morphometric measurements. The approach is focused on using a set of low dimensional projections where the authors can measure the discrepancy between the two distributions of the projections of particles, then assess the performance of an omnibus score for a given class of projections and a corresponding distributional comparison method by comparing the within source and between source score distributions using receiver operator characteristic (ROC) curves. This results in a gross measure of the performance of the score when used for common but unknown source identification problems that commonly arise in forensic applications, such as with IEDs.

New developments in the interpretation of pairwise comparison procedures for this class of forensic applications will also be presented.

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#### **Improvised Explosive Devices, Aluminum Powder, Micromorphometry**



## B160 Learning Algorithms to Evaluate Glass Evidence

Soyoung Park, MS, Center for Statistics and Applications in Forensics, Iowa State University, Ames, IA 50011; Samantha Tyner, PhD, Center for Statistics and Applications in Forensics, Iowa State University, Ames, IA 50011; Alicia L. Carriquiry, PhD\*, Center for Statistics and Applications in Forensics, Ames, IA 50011

**Learning Overview:** After attending this presentation, participants will know about a new approach for analyzing and comparing glass evidence using the elemental concentrations in the glass. Participants will also learn how to access and use the database built by Iowa State University researchers, in collaboration with researchers at the University of Iowa.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting some limitations in the current methods to interpret glass evidence and by proposing an alternative approach to do so.

Glass evidence arises in criminal cases whenever a glass object such as a house window or a car windshield is broken. If glass fragments are recovered from the suspect, the question of interest is whether the fragments on the suspect could have originated from the broken item at the crime scene. With technology such as LA-ICP-MS, it is possible to measure the content of many elements in the glass with high precision. Using this information, researchers have proposed interval-based criteria to compare two glass fragments and decide whether they are chemically indistinguishable.<sup>1,2</sup> Roughly, these approaches use measurements from known fragments to compute univariate intervals around the elemental composition means and then declare that the question fragment has an indistinguishable chemical composition if all mean elemental concentrations fall within those intervals. Guidelines on the application of these methods are given in ASTM E2330 and ASTM E2927.<sup>3,4</sup>

A machine learning approach is proposed to compare two glass fragments for the purposes of classifying them as being indistinguishable (a “match”) or not. The approach computes a similarity score for each pair of fragments using the difference in the content of 18 elements as “features.” The features are combined into a single score via a random forest. The score takes on values between 0 and 1; the higher the score, the stronger the evidence in favor of a “match.”

To construct and test the algorithms, a database of glass fragments has been created. At present, the database contains over 1,500 fragments, obtained from 48 different samples of float glass produced by two different U.S. manufacturers over a period of one month. Each glass sample was broken into fragments and 24 fragments from each sample were randomly selected for analysis. Measurements on each fragment were replicated 5 times, and for 3 fragments in each sample, the number of replicates was increased to 20. Thus, the database that has been created permits estimation of the variability in elemental composition at many different levels: within fragment (between replicates), within sample (between fragments), within manufacturer (between samples), and between manufacturer. Because of the design of the data collection approach, it is also possible to explore whether glass manufactured within the same day, or within the same week is like glass manufactured over a longer period of time in the same manufacturing plant.

It is shown that when using these data combined with the data collected by the proposed algorithm out-performs the interval-based comparison criteria.<sup>1</sup> When applied to data other than the data used to develop the interval-based methods, their performance deteriorates significantly, and result in large numbers of miss-classifications.

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### Glass Evidence, Learning Algorithms, False Positives



**B161 WITHDRAWN**



### B162 Trace Vapor Generator for Explosives and Narcotics (TV-Gen): An Overview

*Susan Rose-Pehrsson, PhD, NRL, Washington, DC; Greg Collins, PhD, NRL, Washington, DC; Michael Malito, Nova Research, Inc., Alexandria, VA; Mark Hammond, PhD, NRL, Washington, DC; Lauryn DeGreeff, PhD, U.S. Naval Research Laboratory, Washington, DC 20002; Christopher J. Katilie, Nova Research, Inc., Alexandria, VA 22308; Michelle Cerreta, PhD, International Forensic Research Institute, Miami, FL 33199; Alison G. Simon, PhD\*, Washington, DC 20375*

**Learning Overview:** After attending this presentation, attendees understand about the Trace Vapor Generator for Explosives and Narcotics (TV-Gen), a system designed by the U.S. Naval Research Laboratory to produce continuous and stable vapor streams for the purpose of validation of trace vapor detectors. They will learn about the system and the characterization of a suite of contraband vapors.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing a method for evaluation of novel sensors and instrumentation using validated vapor streams.

Trace vapor detection of hazardous materials including explosives and narcotics is critical to the protection of military and civilian personnel both in the United States and abroad. The ability to detect trace vapors can enhance identification of explosive devices as well as border protection by preventing smuggling of narcotics or other illegal cargo. Researchers and instrument developers continue to advance trace vapor detection technology through the development of new materials and sensors for hazardous chemicals and vapors. However, uniform, reliable methods of evaluation for new detection systems are limited, making assessments and comparison across the field challenging. Additionally, the generation of explosive and narcotic vapors at reproducible, measurable rates is extremely difficult due to their low vapor pressures.

The U.S. Naval Research Laboratory has designed and constructed the TV-Gen to evaluate new materials, sensors, and instrumentation for hazardous vapor detection. The TV-Gen was developed to generate trace levels (parts per million to parts per quadrillion) of explosive or narcotic vapor. It provides a single output with a dual manifold that can be easily and efficiently switched between clean air and trace hazardous chemicals. Humidity and analyte introduction is made using the custom Pneumatically Modulated Liquid Delivery System (PMLDS) coupled to a perfluoroalkoxy (PFA) total-consumption microflow nebulizer that reproducibly and accurately generates trace vapors of low vapor pressure compounds. TV-Gen also consists of a custom oven capable of being heated up to 130°C, ensuring minimal analyte adsorption to the surfaces of the dual manifold. The manifold can be completely disassembled for thorough cleaning, and the custom control box utilizes computer control for standardized vapor delivery. An online validation system then provides near real-time verification of each analyte over a wide range of concentrations and relative humidities. The TV-Gen has been fully characterized with the following explosives: nitromethane, nitroglycerine, ethylene glycol dinitrate, 2,4,6-trinitrotoluene, pentaerythritol tetranitrate, and hexahydro-1,3,5-trinitro-1,3,5-triazine, as well as with the narcotic methamphetamine. Preliminary work has been completed with cocaine and 3,4-methylenedioxyamphetamine. This presentation will discuss the development of the TV-Gen as well as the characterization of certain explosive vapor streams. The method development process for generating vapors of the three low vapor pressure narcotics mentioned previously will also be presented in detail. These narcotic compounds were selected for initial testing based on vaporization temperature and vapor pressure. Parameters to be discussed include: nebulizer liquid and air flow rates and temperature, manifold temperature, liner type, sample volume, and trapping temperature. For example, using these optimized parameters, vapor generation efficiency for methamphetamine was  $93 \pm 3.3\%$ .

#### Trace Vapor Detection, Hazardous Vapor Generation, Explosives



### **B163 MicroFLOQ®: Collection and Direct Amplification Methods Using the GlobalFiler™ Kit for DNA Recovered From Common Pipe Bomb Components**

*Esiri Tasker, BA\*, Sam Houston State University, Huntsville, TX 77340-6685; Carrie Mayes, BS, Sam Houston State University, Huntsville, TX 77340; Sheree R. Hughes-Stamm, PhD, Sam Houston State University, Huntsville, TX 77340*

**Learning Overview:** The goal of this presentation is to demonstrate that direct amplification using microFLOQ® swabs can be more informative compared to traditional DNA processing by providing more complete STR profiles from challenging, low-quality "touch" DNA samples from common pipe bomb components.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by demonstrating how using microFLOQ® swabs and direct amplification can be advantageous when processing "touch" DNA samples.

Improvised explosive devices (IEDs) such as pipe bombs are often used to cause fear and devastation within communities. When attempting to analyze DNA from recovered pipe bomb fragments, quantities are often limited which can make DNA typing extremely difficult. Amplifying trace amounts of DNA can cause stochastic effects. Effects such as peak height imbalance, allele and/or locus dropout, and failed amplification can render a profile uninterpretable and result in lost investigative leads. Therefore, the efficiency of the initial collection of DNA from challenging items of evidence is important to maximize the amount of DNA available for downstream analysis.

Alternate methods may be better suited for processing "touch" and other challenging DNA samples. Direct amplification bypasses the extraction and quantification steps by placing the collected sample directly into the PCR reaction. This reduces DNA loss, increases the amount of starting template available for amplification, and increases the likelihood of generating more complete profiles. However, direct amplification kits and protocols typically target high molecular weight DNA and are intended to be used for reference samples.

The aim of this study was to optimize the recovery of mock "touch" DNA from common pipe bomb substrates by exploring two swab types (cotton and microFLOQ®) and various direct amplification methods. An epithelial cell suspension (~30 cells or 200 pg DNA) was spiked onto four different pipe bomb substrates (PVC pipe, galvanized steel pipe, electrical tape, and insulated copper wire), and swabbed with either cotton or microFLOQ® swabs. microFLOQ® swabs were placed directly into the PCR reaction for amplification or incubated for 20 minutes at room temperature in 40 µL of TE prior to direct PCR. Cotton swabs were either extracted using the PrepFiler *Express*™ kit on the Automate *Express*™, amplified directly using the GlobalFiler™ PCR Amplification kit, or incubated in 400 µL of TE for 20 minutes prior to amplification.

The results from this study showed that direct amplification of the microFLOQ® swabs resulted in the most complete profiles when amplified directly. The data further support the notion that traditional DNA processing may not be the most suitable method for processing "touch" and low-template DNA samples for STR analysis. In addition, direct amplification with microFLOQ® required the least number of steps, which reduced processing time and decreased the risk of contamination.

#### **MicroFLOQ®, Pipe Bomb, Direct Amplification**



### **B164 Cellular Autofluorescence Signatures for Quantifying Cell Types in Trace Biological Samples and Establishing Age of Evidence**

*Emily Brocato, Virginia Commonwealth University, Richmond, VA 23284; Kate Philpott, JD, Reston, VA 20191; Christopher J. Ehrhardt, PhD\*, Virginia Commonwealth University, Richmond, VA 23284*

**Learning Overview:** After attending this presentation, attendees will learn about cellular autofluorescence profiles and how these signatures can be used to quantitate the abundance of forensically relevant cell types and determine the amount of time between deposition of cell populations and sample collection. Attendees will also learn how flow cytometry and conventional microscopic techniques may be used to rapidly analyze these signatures in biological evidence.

**Impact on the Forensic Science Community:** This research will impact the forensic science community by introducing a new, non-destructive approach for determining source tissue for cell populations in a biological sample as well as the overall age of the evidence. This may provide probative information for many types of biological samples and complement results from standard genetic profiling techniques.

Characterizing the type of cells present in biological evidence and, therefore, the tissue they originated from within the body, can assist with crime reconstructions and downstream DNA profiling methods. Traditionally, case working methods for determining tissue source are based on microchemical reactions targeted toward proteins within bodily fluids, which have limited sensitivity and/or specificity. In contrast, few forensic techniques have utilized morphological or intrinsic biochemical profiles of cells due to the laborious nature of conventional microscopic characterizations or the need for tissue-specific probes which have limited success on compromised samples. Although autofluorescence signatures have demonstrated applications for clinical diagnostics and separating forensic cell mixtures, they have not been investigated as a method to identify cell types or establish the age of a cell population.

Therefore, the goal of this study was to characterize autofluorescence and morphological signatures for cell populations representing four separate tissue sources: buccal, touch epidermal, vaginal, and blood, and then test whether autofluorescence profiles could be used to rapidly differentiate cell types in aged biological samples. Cell populations were collected from a total of 30 individuals, 10 per each tissue type. Additionally, each sample was aged for between 24 hours and more than two months. Measurements from 45 different variables were collected from individual cells in a high-throughput fashion using flow cytometry and then analyzed using multivariate techniques including Discriminant Function Analysis. Results showed that epidermal cells could be distinguished from vaginal and buccal cells with a high degree of classification accuracy, ~94%. Similarly, blood cells could be differentiated from the three epithelial cell types with an accuracy over 97%. Analysis of variable weights indicated that measurements capturing the circularity, aspect ratio, and autofluorescence between 450-650nm of cells were the largest drivers of multivariate differences between tissue types.

Cellular autofluorescence profiles were also observed to change systematically as the sample aged. Specifically, the median intensity of autofluorescence detected between 350-550nm and between 630-680 nm increased incrementally at 24-hour time points between zero and seven days (e.g., ~4500 RFU at 24 hours; ~7500 RFU at 48 hours, ~9000 RFU at 72 h). Additionally, differences were observed between one week and two months that were less systematic with time. Autofluorescence signatures were then used to construct a multivariate framework to predict the time since deposition in an unknown biological sample. Results showed that cell populations were correctly associated with eight different time points between 24 hours and 2 months with an accuracy of ~85%. Ultimately, cellular measurements such as these, which can be obtained non-destructively, may provide probative information for many types of biological samples and can complement results from standard genetic profiling techniques.

**Forensic Biology, Autofluorescence, Serology**





### **B165 Distinguishing Between Fluorinated Surface Treatments of Fibers Using Pyrolysis-Gas Chromatography Plasma-Assisted Reaction Chemical Ionization-Mass Spectrometry (py-GC-PARCI-MS)**

*Michael J. Dolan, Jr., MS\*, Georgetown University, Washington, DC 20057; Robert D. Blackledge, MS, El Cajon, CA 92021; Kaveh Jorabchi, PhD, Georgetown University, Washington, DC 20057*

**Learning Overview:** The goal of this presentation is to demonstrate how a new ion source can be used with pyrolysis-GC/MS (py-GC/MS) to enhance the evidential value of fibers by distinguishing between identical fibers with different fluorinated surface treatments.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of a new tool for discrimination of single fibers based on surface chemical characteristics, currently not attainable using visual and spectroscopic techniques.

The weight of fiber evidence is directly linked to the rarity of a fiber type. Because blue and white cotton are extremely common, their value as evidence is typically extremely limited. However, if these common fibers are coated, either by the owner or by a manufacturer, their value may be significantly increased. One type of surface coating that has been probed for this purpose is fluorinated water and stain resistant coatings. Many people own blue jeans, but fewer people will have jeans that are coated with a water and stain resistant coating. The presence of these coatings can be experimentally determined by examining the contact angle of water and oil. However, there are multiple types of coatings, and distinguishing between these different types on a single fiber has not been successfully achieved except by XPS, which is expensive and often unavailable to forensic laboratories.<sup>1</sup>

Here, a new elemental ion source coupled to pyrolysis GC/MS is presented that has high selectivity and sensitivity for fluorine and other halogens. By using an elemental source matrix effects are significantly reduced. An additional benefit to using an elemental source is that pyrolysis products of the fluorinated coating are easily observed as peaks with  $m/z$  19. The GC is an essential part of this instrument because it provides some structural information of the polymer that is lost by using an elemental source. It does this by separating the fluorine containing pyrolysis products prior to atomization and ionization. By comparing pyrogram relative retention times and normalized intensities the results can be stored in a library that other labs could use for comparison.

To evaluate this new approach, pre-coated cotton fibers as well as cotton fibers applied in the lab were tested. For lab applications the pad-dry-cure method or spraying was employed. For the pad-dry cure method a small swatch of cloth was soaked in the fluoropolymer solution prepared according to manufacturer specifications, then excess liquid was squeezed out and the fabric was dried and cured using a circular IR lamp. For spraying, instructions on the commercial spray bottle were followed. No heating was required for the spray applications. A thread from the treated fabric was then removed and a single fiber separated. The fiber was then cut to 10 mm and transferred to a quartz tube for pyrolysis.

With this new ion source fluorinated coatings have been successfully detected on single fibers. Furthermore, data collected thus far shows the capability of distinguishing at least three different groups of fluorinated coatings by using principal component analysis (PCA). When visually comparing raw pyrograms the differences between these groups are clear. Therefore, py-GC-PARCI-MS is capable of being used to detect and differentiate between different coatings, improving the evidentiary value of fibers that have been coated.

In this presentation a new ion source coupled to pyrolysis GC/MS will be described that has been shown to distinguish between different fluorinated surface treatments on forensically relevant single fibers. This technique could also be potentially used to detect other halogenated coatings such as some dyes, and insect repellents. Applications of this new technique are likely to expand beyond fiber analysis and may find many applications in forensic analysis.

*This project was supported by Award No. 2017-R2-CX-0007, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.*

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#### **Fibers, Pyrolysis, Coatings**



### **B166 The Effect of Ultraviolet Radiation on the Microspectrophotometry (MSP) of Dyed Textile Fibers: Spectral Alteration Categories**

*Meggan B. King, BSc, McCrone Research Institute, Chicago, IL 60616; Sebastian B. Sparenga, MS, McCrone Research Institute, Chicago, IL 60616; Gary J. Laughlin, PhD, Chicago, IL 60616; Patrick Buzzini, PhD\*, Sam Houston State University, Huntsville, TX 77340*

**Learning Overview:** After attending this presentation, attendees will understand about the alterations in spectra collected using ultraviolet/visible (UV-Vis) microspectrophotometry (MSP) that can occur when dyed fibers are exposed to ultraviolet (UV) radiation at different time intervals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information useful to the interpretation of microspectrophotometry (MSP) data in typical casework when unknown fiber specimens and reference fiber samples are collected at different times.

MSP is the method of choice for the comparative examination of dyed textile fibers. Ordinarily, fiber specimens of unknown origin (e.g., fibers from clothing recovered at crime scenes) are compared using the same conditions as reference samples from a suspected source. However, in some instances, reference fibers may be collected at a time shortly, or long, after the recovery of the unknowns. During this lapse of time, one or both sets may undergo fiber dye degradation due to weathering (e.g., exposure to natural sunlight).

In this project, MSP spectral alterations are being studied using a reference collection of 53 different swatches consisting of man-made commercial and man-made custom-dyed fibers. Swatches are being exposed to natural sunlight in Arizona and UV radiation in a light box in the laboratory at McCrone Research Institute in Chicago for what will be a total duration of 18 months. MSP is proposed to measure the effect of ultraviolet radiation on color from a variety of fiber and dye types. Samples include common fiber types, such as polyester, nylon, and acrylic, with different color dyes typical for each fiber type. The current phase of this study (July 2018) focuses on the types of spectral alterations that can occur when dyed fibers have been exposed to UV radiation for 8, 16, and 24 weeks.

Because MSP also uses visible and UV radiation, a separate study of instrument-induced photobleaching of 60 minutes, with spectra collected every 60 seconds, was also carried out for each sample to study any degradation in the collected spectra that may occur during routine MSP analysis. The goal was to detect sets of spectra for a given week (8<sup>th</sup>, 16<sup>th</sup>, 24<sup>th</sup>) that do not fit in the range of variation of the spectra collected without previous UV exposure (T0). The first spectral deviations from the range of variation of spectra collected at T0 and the type of initial spectral alterations were recorded; photomicrographs were collected during analyses to compare visual fiber dye-color fading and spectral deviations for a given time. After MSP instrument-induced photobleaching, it was noted that all except one sample exhibited spectral deviations. Two samples could not be assessed due to their large intra-source spectral variation. The results show that different times of occurrence for the onset of spectral alterations were measured for the different samples. These spectral alterations were observed either in the visible range only, in the short UV range only, or in both the visible and UV spectral ranges. Spectral alterations were observed and grouped into six different categories: out-of-range (20 samples), band shifts (10 samples), band flattening (six samples), bump formation (five samples), bands fusion (one sample), and combinations of these (eight samples).

From the reference collection of 53 samples, for fifteen of them no spectral differences were observed between MSP captured at T0 and subsequent weeks up to 24 weeks. However, spectral alterations were observed for 32 samples. Twenty samples exhibited differences in spectra collected at zero and eight weeks of UV exposure. Spectral alterations were first noticed after 16 weeks for seven samples. Spectral changes were observed for five samples after a UV exposure of 24 weeks. It was not possible to determine the occurrence of spectral alterations for six samples due to the high intra-source variation of spectra collected after various weeks.

Finally, with very few exceptions and from the photomicrographs collected in conjunction with MSP analysis, photobleaching of the fibers was not visible together with the observed spectral alterations, indicating that spectral alterations generally precede visually detectable photobleaching. This is not in agreement with the simple observation of the textile fiber swatches that appear noticeably bleached after weeks of UV exposure.

*This research was funded by the National Institute of Justice (Award No. NIJ-2016-DN-0145).*

#### **Trace Evidence, Fiber, Microspectrophotometry**



### **B167 The Discrimination and Identification of Fiber Samples Using Raman Microspectroscopy**

*Sergey Mamedov, PhD\*, HORIBA Scientific, Piscataway, NJ 08854*

**Learning Overview:** After attending this presentation, attendees will understand Raman microspectroscopy in applications to fiber analysis discrimination and identification, which are important elements in forensic identification of these materials.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a key aspect of fiber analysis and an example of a practical application of Raman microspectroscopy to fiber identification.

Raman spectroscopy and infrared spectroscopy are complementary techniques, often used for the identification of compounds. Raman spectroscopy offers several advantages over infrared spectroscopy. Raman spectroscopy is a light scattering technique whereby light from a laser interacts with a sample producing scattered light of different wavelengths. The scattered light, which is specific to a particular compound, is directed to a detector enabling chemical identification. Raman analysis has been recognized to have potential for solving a wide variety of problems associated with forensic science. Early motivation was to identify substances and contaminants that appeared in crime scene evidence and manufactured products. Nevertheless, it was quickly applied to all types of material analyses.

Raman microspectroscopy is very applicable in the field of forensics. It uses a technique that offers a non-destructive and non-contact method of analysis. Only a small amount of sample is required and little or no sample preparation is necessary. It allows for trace analysis, whereas sampling can be done directly through transparent evidence bags and packaging, such as glass and plastics. It covers a wide spectral range from  $10\text{ cm}^{-1}$  to  $4000\text{ cm}^{-1}$ , making the technique ideal for the identification of both organic and inorganic substances, which includes fibers, drugs, pharmaceuticals, explosives, inks, paint etc. Raman microspectroscopy also allows to identify the components of inhomogeneous samples and to obtain automated high definition Raman mapped images.

To aid law enforcement personnel and the public at large, investigations have been geared toward the ability of Raman microspectroscopy to identify a variety of polymers used in fibers. This is very important, as the presence of fibers at a crime scene has often been instrumental in the process of solving crime. The capability of Raman spectroscopy to differentiate between fibers of similar chemical structure will be demonstrated. “Fingerprints” of nylon 6, Kevlar, polystyrene, PET, poly-propylene, and some other fibers along with different types of nylon (nylon 6, nylon 6/6, nylon 12, and others) will be highlighted in this paper, as well as the ability to identify fiber mounted on substrate.

Spectral data of the fibers was collected using 532 nm, 633 nm, and 785 nm laser excitations. Comparison of the Raman spectra of the fibers taken with different excitation wavelength will be discussed. It will be shown that search can provide quick identification of materials whose spectra have been collected in a library or just matched to suspect material samples.

#### **Raman, Fibers, Identification**

### B168 The Identification of Phencyclidine (PCP) and Designer PCP Analogs Using Microcrystalline Tests Followed by Raman Microspectroscopy

Matthew Quinn\*, Cedar Crest College, Allentown, PA 18104-6196; Lawrence Quarino, PhD, Cedar Crest College, Allentown, PA 18104; Monica Joshi, PhD, West Chester University, Department of Chemistry, West Chester, PA 19383; Thomas A. Brettell, PhD, Cedar Crest College, Allentown, PA 18104

**Learning Overview:** After attending this presentation, attendees will understand a new protocol that is fast and confirmatory for the identification of phencyclidine (PCP) and several designer PCP analogs that simultaneously incorporates microcrystalline tests and Raman microspectroscopy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the effectiveness of a novel drug analysis technique, which has the potential to be used in forensic laboratories for the identification of new designer drugs.

Designer drugs are compounds being synthesized and distributed to circumvent drug schedules and the legal system. It is the duty of a forensic scientist to identify the chemical structures of these compounds in today's evolving drug market. This has created an immense challenge in crime laboratories across the world.<sup>1-3</sup> Color tests are widely used for preliminary screenings and are most often paired with mass spectrometry, the current gold standard for confirmatory drug analysis.<sup>4</sup> These techniques have proven to be consistent and reliable when analyzing traditional drugs of abuse like cocaine, heroin, and amphetamines. However, they are not universal, and the emergence of designer drugs has posed a hindrance on the traditional analytical procedures. Due to accreditation standards, a lab must go through rigorous method development and validation to analyze new drug compounds within their standard operating procedures. Understanding the chemistry of a new drug takes time and resources. This issue has sparked a need for re-evaluation of standards for the analysis of new emerging drugs, especially regioisomers of traditional drugs.

In this study, PCP and twelve designer PCP analogs (tenocyclidine, rolicyclidine, benocyclidine, phencyclamine, eticyclidine, 3-methoxy phencyclidine, 4-methoxy phencyclidine, PCEEA, PCMPA, 3-methoxy eticyclidine, methoxetamine, diphenidne) were subjected to microcrystalline tests followed by Raman microspectroscopy on the successfully grown microcrystals. The method proved reproducible through replicate examinations over several days. A microcrystalline test method for each compound was developed using exact concentrations and volumes to eliminate the ambiguity of previous microcrystalline testing. Additionally, a Raman microspectroscopy method was developed to ensure high quality spectra and the ability to identify the original compound. The combination of these two analytical techniques adhere to the recommendations described by the Scientific Working Group for the Analysis of Seized Drugs.<sup>5</sup> A recent study has demonstrated the viability of utilizing microcrystalline tests in tandem with Raman microspectroscopy for the identification of designer drugs.<sup>6</sup>

Microcrystal properties such as shape, habit, time of growth, color, retardation colors, type/angle of extinction, and sign of elongation (when applicable) were observed and documented. These optical properties were proven effective in distinguishing structurally similar compounds. Using pictures and descriptions, microcrystalline test results for PCP and designer PCP analogs previously documented will be presented here in a clear, more detailed fashion. Results for compounds not yet analyzed in literature are described in the same manner.

Analysis with a Raman microscope was able to provide structural information on the microcrystals. A library containing pure drug spectra was used to compare drug microcrystal spectra and ultimately identify the original compound. Further spectral comparisons indicated peak shifts along with the addition or subtraction of specific peaks for each microcrystal.

This presentation will demonstrate the utility of microcrystalline tests followed by Raman microspectroscopy for the identification of PCP and designer PCP analogs. While this research focuses on a specific structural class, the analytical procedure can be applied to all other classes for fast and confirmatory identification.

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#### Designer Drugs, Microcrystalline Tests, Raman microspectroscopy



### **B169 Field Identification of Kratom by Portable Gas Chromatography/ Mass Spectrometry (GC/MS) Instrumentation**

*Zachary Lawton, MS, PerkinElmer, Inc., Shelton, CT 06484-4794; Sarah Goda, BS, University of New Haven, West Haven, CT 06516; Peter Massey, MS, University of New Haven, West Haven, CT 06516; Brooke W. Kammrath, PhD\*, University of New Haven, West Haven, CT 06516*

**Learning Overview:** After attending this presentation, attendees will understand how mitragynine, the active component of Kratom, can be positively identified in the field by portable gas chromatography/mass spectrometry (GC/MS) instrumentation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the first method for the on-scene identification of Kratom, which is valuable because other field deployable methods such as color tests and vibrational spectrometers are unable to identify this opioid.

Kratom is a relatively new “legal high” in the United States that is easily purchased over the internet, and its federal legality is under considerable debate. It has been linked with at least 44 deaths which have triggered significant attention by the FDA and DEA. Kratom comes from the *Mitragyna speciosa* leaves which is part of the *Rubiaceae* family native to South East Asia. Originally the plant was ingested for its stimulant properties, and it is advertised today as a concentration booster, a workout enhancer, a replacement for opioid painkillers and as a way to treat opioid addiction. The main active compound in kratom is mitragynine which in low doses binds to the delta-opioid receptors where it acts as a stimulant, but as the dose increases it binds to the mu-opioid receptors and becomes a sedative. It is in the same category as morphine but is 13 times more potent of an agonist for the receptors, thus it is similarly addictive. Consequently, in February 2018, the FDA determined that kratom was more than just a plant and classified it as an opioid. Although it has not yet been scheduled by the DEA, its ban appears to be imminent with this new FDA classification.

Law enforcement officers commonly rely on the field identification of illicit drugs for arrests. The most commonly employed field test is a presumptive colorimetric test called the Narcotics Identification Kit (NIK) which relies on a reaction with the provided reagents to produce characteristic color changes. To date, no colorimetric test has been found to identify Kratom or its active compounds. Infrared and Raman spectrometry are also not suited for the identification this opioid due to the complexity of the plant matrix. In the lab, chromatographic tests (GC/MS and HPLC/MS) are used for the confirmatory identification of mitragynine in Kratom. A gap has thus been identified because there is a need for the on-scene identification of Kratom by the law enforcement community. Field deployable GC/MS instruments are of considerable interest for law enforcement and hazmat teams, with applications for explosive and general unknown identifications, illicit drug testing, and clandestine lab investigations. This research reports on the positive identification of mitragynine in Kratom by portable GC/MS instrumentation. The Torion T-9 portable GC/MS using SPME sampling with direct injection via thermal desorption successfully detected and identified mitragynine in 14 samples of kratom: 10 powders, 3 pills, and an oil extract.

#### **Portable GC/MS, Kratom, Mitragynine**



### B170 2019 Update From the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)

*Sandra E. Rodriguez-Cruz, PhD\*, Drug Enforcement Administration, Vista, CA 92081*

**Learning Overview:** The goal of this presentation is to provide the forensic chemistry (drug analysis) community with the most up-to-date information about resources and documents available from the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing their awareness of latest developments in the seized drug discipline, as well as new documents and resources that can enhance and complement their practices.

The SWGDRUG was formed in 1997 in a joint effort between the United States Drug Enforcement Administration (DEA) Office of Forensic Sciences and the Office of National Drug Control Policy (ONDCP). SWGDRUG works to improve the quality of the forensic examination of seized drugs and to respond to the needs of the forensic community by supporting the development of internationally accepted minimum standards, identifying best practices within the international community, and providing resources to help laboratories meet these standards. This presentation will provide attendees with information on SWGDRUG activities during the past year.

Core committee members are currently working on revisions to PART IIIB of the SWGDRUG Recommendations. The purpose of PART III B is to recommend minimum requirements for the forensic identification of seized drugs or chemicals. A reliable and scientifically supported identification of a drug or chemical depends on the use of an appropriate analytical scheme by competent analysts in a quality-controlled process. PART III B addresses the overall selection of techniques, the rationale behind their categorization, and emphasizes the need to develop robust analytical schemes dependent on the scenario at hand or jurisdictional application.

A new supplemental document SD-7 (Construction of an Analytical Scheme) is also undergoing development. The purpose of this supplemental document is to provide guidance to practitioners on the construction and implementation of appropriate analytical scheme as required by SWGDRUG Recommendations PART IIIB. It will include more than a dozen examples of analytical schemes applicable to many jurisdictions. This annual update will include some of those examples, their rationale, limitations, and applicability.

SWGDRUG committee members are also working on revisions to PART IVB (Validation of Analytical Methods) of the Recommendations. Revisions will include additional background information and clarifications on the performance characteristics to be evaluated during the validation of both qualitative and quantitative methods. Furthermore, the currently existing Supplemental Document SD-2 (Validation of Analytical Methods) is also being revised and expanded to better assist seized-drug practitioners during method validation activities. Additions will include examples of qualitative method validation plans and studies for color test, gas chromatography-mass spectrometry (GC/MS) and infrared (IR) spectroscopy, as well as guidance on retrospective validations and method modifications.

This presentation will also summarize recent updates on SWGDRUG resources like the MS library, IR library, and Drug Monographs, available to practitioners via the SWGDRUG website ([www.swgdrug.org](http://www.swgdrug.org)). SWGDRUG continues its partnership with the National Institute of Standards and Technology (NIST) to verify the quality and reliability of the SWGDRUG MS Library to provide valuable and reliable resources to the community. Drug Monographs also continue to be added and disseminated and this highly-used resource has also been enhanced to allow searches and sorting by name, nominal mass, and base peak.

The SWGDRUG core committee includes representatives from federal, state and local law enforcement agencies in the United States, Canada, Brazil, Austria, Switzerland, Australia, and Singapore. The following international forensic organizations are represented: the European Network of Forensic Science Institutes (ENFSI), the Academia Iberoamericana de Criminalística y Estudios Forenses (AICEF), the Asian Forensic Science Network (AFSN), and the United Nations Office on Drugs and Crime (UNODC). Core committee members also include forensic science educators and representatives from forensic science organizations across the United States, the American Society of Crime Laboratory Directors (ASCLD), the American Society for Testing and Materials (ASTM International), the National Institute of Standards and Technology (NIST), and the Federal Bureau of Investigations (FBI).

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#### Criminalistics, Seized Drugs, SWGDRUG



### B171 The Identification of a Novel Fragmentation Pathway of Synthetic Cathinones

*J. Tyler Davidson, MS\**, West Virginia University, Morgantown, WV 26505; *Zachary J. Sasiene, BS*, West Virginia University, Morgantown, WV 26506; *Younis F. Abiedalla, PhD*, Auburn University, Auburn, AL 36830; *Randall Clark, PhD*, Auburn University, Auburn, AL 36849; *Glen P. Jackson, PhD*, West Virginia University, Morgantown, WV 26506-6121

**Learning Overview:** After attending this presentation, attendees will know about a novel fragmentation pathway observed through protonated tandem mass spectra of synthetic cathinones. Attendees will also learn the concepts of Multistage Mass Spectrometry ( $MS^n$ ) and isotopic labeling for structural elucidation and will gain a sense of the similarities and differences between the fragmentation behavior of drugs that are fragmented under different conditions, such as Electron Ionization (EI), Electrospray Ionization with Collision-Induced Dissociation (ESI-CID-MS), and Direct Analysis in Real-Time (DART) ionization with triple quadrupole mass spectrometry.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of the fragmentation of synthetic cathinones under different ionization and fragmentation conditions. Additionally, the identification of a novel fragmentation pathway(s) for the generation of the tropylium ion ( $m/z$  91) and the methylenedioxy derivative ( $m/z$  135) with the use of  $MS^n$  and isotopic labeling helps clarify confusion about the origin of these ions.

**Hypothesis:** The central hypothesis is that the combination of  $MS^n$  mass spectrometry and isotopic labeling will result in the identification of an unexplained fragmentation pathway(s) for the generation of the tropylium ion for synthetic cathinones. The authors hypothesize that the ionization conditions, fragmentation conditions and substituent groups will affect the propensity for the formation of the tropylium ion along this novel pathway.

**Methods/Results:** Analyses involved the identification of a novel fragmentation pathway for the generation of the tropylium ion ( $m/z$  91) or methylenedioxy derivative ( $m/z$  135) using a combination of  $MS^n$  mass spectrometry and isotopic labeling. The compounds PV8,  $\alpha$ -PVP,  $^{13}C\alpha$ -PVP (carbonyl carbon labeled), 3,4-MDPV, and 3,4-MDPV-d8 (pyrrolidine labeled) were analyzed using: (1) a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer operated with both ESI and DART ionization sources, (2) a Thermo Scientific LTQ Velos Pro with HESI ionization source, and (3) an Agilent Technologies 7890B GC/5977A MS.

Preliminary results indicate that ESI and DART ionization sources produce similar tandem mass spectra, because both ion sources start with intact protonated molecular ions. Tandem mass spectra, either from in-source CID, beam-type CID or ion trap CID show the tropylium ion, or corresponding methylenedioxy derivative, in all samples analyzed. However, under traditional EI fragmentation, the abundance of the tropylium ion is negligible. The DART and ESI data were collected on the triple quadrupole using in-source CID to produce pseudo  $MS^3$  spectra. However, pseudo  $MS^3$  spectra were not able to definitively prove the mechanism proposed through this work, so  $MS^n$  was performed using the LTQ Velos Pro linear ion trap.

The proposed mechanism begins with the loss of the pyrrolidine ring from the  $[M+H]^+$  precursor. A gamma hydrogen shift leads to a carbon skeleton rearrangement involving a cyclobutone ring fused with the benzene ring. The bicyclic structure then rearranges to eliminate a CO neutral and produce the tropylium ion. Particularly novel is that when the CO neutral is lost, the oxygen from the carbonyl group appears to be equally likely to leave with the original carbonyl carbon or with the carbon atom adjacent to it. These pathways have been proven through  $^{13}C$  isotopic labeling and the observation of tropylium ions at both  $m/z$  91 and  $m/z$  92. The mechanism is observed for  $\alpha$ -PVP, PV8, and 3,4-MDPV.

#### Fragmentation Mechanisms, Isotope Labeling, Multistage Mass Spectrometry ( $MS^n$ )



**B172 WITHDRAWN**





### B173 The Development of an *In Silico* Mass Spectral Library

Janelle D.S. Newman, MD\*, MRIGlobal, Gaithersburg, MD 20878; Eileen B. Eubank, MFS, MRIGlobal, Gaithersburg, MD 20878; Jeremy Zehr, MS, MRIGlobal, Gaithersburg, MD 20878; Joseph A. Trimboli, PhD, Rockville, MD 20850

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**Learning Overview:** The goal of this presentation is to provide forensic practitioners with a tool for rapidly and inexpensively down-selecting novel fentanyl standards for synthesis and purchase.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing forensic practitioners with a tool for rapidly and inexpensively down-selecting novel fentanyl standards for synthesis and purchase.

The abuse of opiates, in particular those in the fentanyl family, is a growing epidemic. Because fentanyl and its derivatives (fentanyls) are readily synthesized, a serious analytical challenge has developed. Much like bath salts and other synthetic cannabinoids, small changes in the structure can make the producers of these compounds more difficult to prosecute. Furthermore, these changes can hinder the efforts of forensic drug chemists and toxicologists to identify samples. A major challenge with identifying emerging fentanyls and other novel psychoactive substances (NPS) is the lack of available standards and their associated mass spectra.

To address this challenge, the authors developed a proof-of-concept, *in silico* mass spectral library for fentanyls. This library contains simulated spectra of as-yet uncharacterized fentanyl derivatives, which facilitates rapid identification of emerging fentanyls. The library contains mass spectra generated using ACD/Labs Spectrus Processor and MS Fragmenter software packages. The resulting theoretical fragmentation patterns were used to create a searchable database based in Microsoft Excel. The process began by drawing chemical structures for reasonable, but uncharacterized, fentanyls. The software package then generated mass spectra by fragmenting the structure according to all allowable fragmentation pathways based on mass spectral fragmentation rules. These fragments comprise the *in silico* spectrum for a compound and are included in the library database.

As an initial test, mass spectra were generated for 15 commercially available fentanyls using the software. Experimental mass spectra were obtained for these compounds by analyzing chemical standards with GC/MS. The experimentally obtained spectra were used as challenge spectra for the database. To evaluate the library, the ten most dominant mass fragments from the GC/MS spectrum were searched against the database. The total number of mass spectral fragments that corresponded with the theoretical mass spectrum in the *in silico* library were recorded, and candidate library matches were determined. The candidate matches were placed into tiers according to the number of theoretical fragments found to match the experimental fragments. The candidates with the largest number of matching fragments were placed into Tier 1. Among the 15 challenge compounds, 12 appeared in Tier 1. Additionally, 12 of the challenge compounds had fragment matches to nine or more theoretical ions.

Library compounds with the highest number of matching fragments can be considered structural candidates for the analysis of unknowns. This can enable a laboratory to acquire novel fentanyl standards using a simple selection process. In addition, this allows an analyst to rapidly evaluate candidate structures without the need for time-intensive structural elucidation studies.

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#### Fentanyls, GC/MS, Library



### B174 A Confirmatory Method for the Analysis of 30 Fentanyl Analogs Using Gas Chromatography/Mass Spectrometry (GC/MS)

Delilah DeWilde, BS\*, Arvada, CO 80004; Matthew R. Wood, PhD, Ocean County Sheriff's Department, Toms River, NJ 08753; Thomas A. Brettell, PhD, Cedar Crest College, Allentown, PA 18104; Thomas H. Pritchett, MS, Allentown, PA 18104

**Learning Overview:** After attending this presentation, attendees will understand the results of various optimizations in GC to improve resolution between fentanyl standards and the importance of accurate reporting of seized drugs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an optimized GC/MS method that can identify 30 fentanyl analogs with improved resolution. The overall run time of 22.33 minutes is practical for forensic use of large amounts of seized-drug casework.

Forensic drug chemists are responsible for reporting the composition of commonly seized drugs. The recent opioid epidemic has resulted in many commonly seized drugs such as heroin, cocaine, and marijuana being observed laced with fentanyl and fentanyl analogues. The Centers for Disease Control and Prevention (CDC) reported in 2016 that opiate related drugs make up 66% of drug related deaths and fentanyl is an increasing source of these drug related overdose deaths.<sup>1,2</sup> The fentanyl epidemic includes fentanyl analogues that are being synthesized in clandestine laboratories, some of which are still not considered illegal due to lag time for legislation. The potency of each analogue varies; fentanyl is estimated to be one 100 times more potent than morphine while an analogue such as carfentanil is estimated to be 10,000 times more potent than morphine.<sup>3</sup> In order to report the presence of fentanyl, drug chemists take steps involving initially performing a qualitative presumptive test. This first test is critical for efficiency but only detects drug classes.<sup>4</sup> The next step is to perform a validated confirmatory test to identify the analyte(s) of interest. Drug chemists typically use gas chromatography/mass spectrometry (GC/MS) to identify drugs.

A confirmatory method for the analysis of 30 fentanyl analogues using GC/MS has been developed. The fentanyl standards included in optimizing this method were fentanyl, crotonyl, acetyl fentanyl, butyryl fentanyl, para-fluorofentanyl, meta-fluorofentanyl, ortho-fluorofentanyl, cis-3-methyl fentanyl, trans-3-methyl fentanyl, para-fluorobutyryl fentanyl, meta-fluorobutyryl fentanyl, ortho-fluorobutyryl fentanyl, acryl fentanyl, valeryl fentanyl, isobutyryl fentanyl, carfentanil, ocfentanil, cyclopropyl fentanyl, alfentanil, sufentanil, remifentanil, W-15, 4-ANPP, para-methoxybutyryl fentanyl, thiofentanyl,  $\beta$ -hydroxythiofentanyl,  $\alpha$ -methyl fentanyl,  $\beta$ -methyl fentanyl, and furanyl fentanyl. This method utilized a gas chromatograph outfitted with two FID detectors and coupled with a single quadrupole mass spectrometer to improve resolution between fentanyl standards utilizing a split injection with a split ratio of (10:1). The preferred GC/MS conditions used an initial temperature of 60°C with an initial hold time of 1.00 minute, the first ramp rate at 30°C/min to 250°C, and then a second ramp rate at 4°C/min to 300°C with a final hold time of 2.50 minutes. The inlet temperature was 280°C and the injection volume was 1  $\mu$ L. This development also investigated the employment of complementary dual columns. The primary column employed was comprised of a 5% diphenyl: 95% dimethyl polysiloxane stationary phase (30 m x 0.25 mm x 0.25  $\mu$ m), the other two columns utilized for analysis were a 5% diphenyl: 95% dimethyl polysiloxane column optimized for amines (30 m x 0.25 mm x 0.5  $\mu$ m) and a trifluoropropylmethyl polysiloxane column (30 m x 0.25 mm x 0.25  $\mu$ m). The columns were compared using calculated linear retention indices. Both columns with a 5% diphenyl: 95% dimethyl stationary phase behaved similarly, with resolution between fentanyl standards remaining preserved using both columns. The column with a trifluoropropylmethyl stationary phase provided complementarity data for standards containing fluorine compounds that originally had elution times overlapping with non-fluorinated compounds using the primary column. The validation process of this method included three sets of six serial dilutions of each standard run on five different days. Stock solutions of mixtures were analyzed as well.

The development of a confirmatory method using GC/MS for the analysis of fentanyl analogues has provided contributory insight for the implementation of a split method, an intricate oven temperature ramp rate, and results from atypical columns for the use of drug analysis. The procedure can be utilized to accurately identify 30 different fentanyl analogues. The method results can be used as a reference using linear retention indices as well as retention times.

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#### Forensic Drug Chemistry, Fentanyl Analogs, Gas Chromatography/Mass Spectrometry



### B175 A Forensic Sample Comparison of Heroin by the Analysis of Its Elemental Composition

Joshua S. DeBord, PhD\*, North Louisiana Crime Lab, Shreveport, LA 71103; Ruthmara Corzo, Florida International University, Miami, FL 33199; Jose R. Almirall, PhD, Florida International University, Miami, FL 33199

**Learning Overview:** After attending this presentation, attendees will understand the methods of sample preparation, instrumental analysis and statistical tools required to perform forensic comparisons of heroin evidence. The goal is to provide a simple means of associating related samples of drugs, as well as discriminating between unrelated samples on the basis of the trace elemental profiles in the evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a means to chemically associate two or more pieces of drug evidence, making possible the establishment of evidence linkages on a micro-scale between a user and a dealer or on the macro-scale between a trafficker and producer.

The goal of this research was to aid in the fight against the heroin and opioid epidemic by developing new methodology for performing forensic sample comparisons of seized heroin evidence. Quantitative elemental data from 120 samples, 30 from each of the four major heroin-producing regions in world (Mexico, South America, Southeast Asia and Southwest Asia), were compared to assess the rate of discrimination (5400 total comparisons).

Forensic sample comparison of illegal drug evidence can be used to associate samples of drug taken at different points of seizure. The development and performance of a statistical means of conducting pair-wise comparisons of heroin samples based on their elemental compositions will be discussed during this presentation. A profile of 11 isotopes,  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{27}\text{Al}$ ,  $^{51}\text{V}$ ,  $^{52}\text{Cr}$ ,  $^{55}\text{Mn}$ ,  $^{66}\text{Zn}$ ,  $^{88}\text{Sr}$ ,  $^{90}\text{Zr}$ ,  $^{111}\text{Cd}$  and  $^{137}\text{Ba}$  was determined to be most useful in correctly associating as well as discriminating between heroin samples in forensic pairwise comparisons. Heroin samples, provided by the U.S. Drug Enforcement Administration Special Testing and Research Laboratory, were prepared by microwave-assisted acid digestion and quantitatively analyzed by inductively coupled plasma-mass spectrometry to measure the elemental abundance within each sample. Both the instrumentation and skills required to perform the analysis are readily available or within the budgetary capacity of full-service crime labs.

Using a match criterion of  $\pm 3$  standard deviations about the mean, only 14 of the 5,400 possible comparison pairs were not discriminated, resulting in a discrimination rate of 99.7%. For determining the rate of correct associations, three replicates of 24 duplicate samples were prepared and analyzed on separate days. Only one of the 24 correct pairs were not associated, resulting in a correct association rate of 95.8%. Type 1 error rates (false exclusion) were found to be 4.2% and type 2 error (false inclusion) rates were 0.03%.

Furthermore, a set of unknown samples suspected of having commonality were compared to test the performance of the method with simulated casework samples. Several of the unknown samples were found to be indistinguishable. Sample to sample similarity in the set of unknown samples was also demonstrated with unsupervised multivariate statistical analysis. This is the first known work to report a method for performing pair-wise forensic sample comparison of heroin by targeting elemental impurities. It is also the first to use authentic heroin samples to develop and test the method and report error rates using a large test set. The availability of a method in which samples can be prepared, analyzed, and compared in less than 24 hours with no necessary chemical derivatizations nor separations is expected to be of great use to forensic drug chemists.

Following this presentation, the audience will be informed of the methods of sample preparation, instrumental analysis and statistical analysis for performing forensic sample comparisons of heroin evidence. In addition, the methods for sample preparation and analysis have been validated for both heroin and SRM NIST 1570a, trace elements in spinach leaves. This suggests that the method of performing sample comparisons is amenable to other small molecule drugs as well as vegetable material, both of which are commonly analyzed by forensic drug chemists.

*This research was supported by the Technical Support Working Group and Counter-Terrorism Technical Support Office [TSWG Task IS-FI-4174]. The authors wish to especially thank Sini Panicker and the Drug Enforcement Administration (DEA) Special Testing and Research Laboratory for sponsoring this effort and providing samples and insight into the research.*

#### Forensic Comparison, Heroin Evidence, Trace Elements



### **B176 Medible Testing: The Preparation of Matrix-Matched Calibrators and Controls for Forensic Analysis**

*Kathryn L. Orton, BS\*, Virginia Commonwealth University, Richmond, VA 23220; Casey Spencer, BS, Virginia Commonwealth University, Richmond, VA 23284-3079; Jean A. Heneks, BS, Richmond, VA 23221; Rachel B. Fielden, MD, Office of the Chief Medical Examiner, Richmond, VA 23219; Justin L. Poklis, BS, Virginia Commonwealth University, Richmond, VA 23219-0613; Carl E. Wolf, II, PhD, Virginia Commonwealth University-Health, Richmond, VA 23298-0165*

**Learning Overview:** After attending this presentation, attendees will understand how to prepare matrix-matched calibrators and controls for analysis of edible marijuana products.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a method to prepare matrix-matched materials that are commercially unavailable.

**Background/Introduction:** In the last decade, the number of states in the United States that have legalized marijuana for both medical and recreational use has risen to 29. Marijuana edibles, or "medibles" are typically packages of candy or baked goods consumed for medicinal as well as recreational use. These products contain the major psychoactive drug in marijuana (delta-9-tetrahydrocannabinol (THC)) and/or cannabidiol (CBD), as well as other cannabinoids that have reputed medical and recreational properties. Federally, the U.S. Drug Enforcement Administration (DEA) has classified marijuana as Schedule I substance, and the only legal formulation of THC is Marinol<sup>®</sup>, which is regulated by the U.S. Food and Drug Administration (FDA). Medibles are regulated by the states in which they are legal rather than the federal government, and therefore there is no standardized method for preparation or analysis of these matrices. The three most common medible matrices were used to prepare matrix matched calibration and control materials (high fiber (brownies), high sugar (gummies), and high fat (dark chocolate)).

**Objective:** To prepare matrix-matched high fiber, high sugar, and high fat materials for forensic analysis of medibles.

**Methods:** Calibration materials were prepared by fortifying 25 mg of each prepared drug-free matrix to the following cannabinoid concentrations (0.8, 1.6, 4, 8, 16, 40, and 80 mcg/g). Control materials were prepared by fortifying drug-free matrix before preparation (i.e., baking, mixing, and tempering) to the following cannabinoid concentrations (0.8, 2.4, 24, and 60 mcg/g). The high fiber matrix was prepared using commercial brownie mix, eggs, and oil. The brownies were baked in a lab oven at 300°F, until done. The high sugar matrix material was prepared using a mix of commercial flavored gelatine with no preservatives, unflavored gelatin, and water. The gummy mix was heated in a microwave, then molded, and chilled, using a previously presented method. The high-fat matrix was prepared using cacao butter, cocoa powder, oil, honey, and vanilla. Several recipes were evaluated for ease of preparation and palatability. The ingredients were melted in a double-boiler and tempered the following day. The concentrations were chosen based on the classification of a serving of THC in states that have legalized marijuana.

**Results:** The high fiber matrix should be baked in mini-muffin or brownie bite tins < 300°F to maintain the stability of the cannabinoids and reduce the baking time needed. The high sugar matrix should be heated only sufficiently to dissolve the sugar matrix and molded soon after dissolution. The high fat matrix should be tempered properly to eliminate the formation of fat blooms.

**Conclusion:** Methods for the preparation of high fiber, high sugar, and high fat matrix-matched materials was determined. Due to unavailability of commercial matrix-matched materials, these methods are available for the preparation of medible materials for future work.

*This project was supported by the National Institute of Justice (NIJ) Research and Development in Forensic Science for Criminal Justice Purposes Grant 2017-R2-CX-0029.*

#### **Cannabinoids, Medibles, Matrix-Matched**



### **B177 A Comparison of Gas Chromatography/Infrared Spectroscopy (GC/IR) and Gas Chromatography/Mass Spectrometry (GC/MS) Methods for the Identification of Isomeric Synthetic Drugs**

*Randall Clark, PhD\**, Auburn University, Auburn, AL 36849; *Lewis W. Smith, BS, Forensic Spectral Research, Bridgeton, NJ 08302*; *Younis F. Abiedalla, PhD, Auburn University, Auburn, AL 36830*

**Learning Overview:** After attending this presentation, attendees will understand the role of gas chromatography/infrared spectroscopy (GC/IR) and gas chromatography/mass spectrometry (GC/MS) for the specific identification of individual regioisomeric substances from the synthetic cannabinoids, cathinones, and N-BOMe drug categories.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by focusing on vapor phase infrared spectroscopy as a confirmatory method of identification for regioisomeric synthetic drugs having equivalent mass based analytical signatures.

The overall goal of this study is to provide an analytical framework for the identification of isomeric forms of synthetic drugs. This presentation will focus on vapor phase infrared spectroscopy (GC/IR) as a confirmatory method of identification for regioisomeric synthetic drugs having equivalent mass based analytical signatures. Examples from several drug categories will be used to illustrate the advantages of GC/IR in the identification of regioisomeric synthetic drug substances, synthetic intermediates, and precursor chemicals.

The relentless development of new designer substances of synthetic origin creates challenges in forensic drug identification. The availability of a wide variety of precursor substances can yield numerous isomeric substances in several drug categories. Issues of regioisomerism are prominent in the cannabinoids, cathinone derivatives, N-methoxybenzyl-phenethylamines (N-BOMe) compounds as well as most other synthetic drug categories. Regioisomeric substances have the identical elemental composition, nominal and exact masses and in many cases yield regioisomeric fragment ions of equivalent elemental composition (equal mass).

The vapor phase infrared spectra are obtained at the elevated temperature of the GC transfer line under conditions equivalent to those used in GC/MS experiments. The higher temperature vapor phase removes many barriers of molecular conformational and rotational restraints yielding fewer individual peaks and broader bands. However, these vapor phase conditions yield spectra free from intermolecular interactions and matrix effects since the ultra-high-purity helium of the mobile phase is the only possible interacting species.

Regioisomeric forms of synthetic substances of equivalent elemental composition and yielding regioisomeric fragment ions of equal elemental composition present unique challenges in forensic drug identification using mass based analytical methods. The mass spectra for many regioisomeric substances are essentially identical and provide no unique ions for structural differentiation. Vibrational spectroscopy however measures small energy differences based on rotation/vibration amplitudes for individual molecular bonds. The interaction between neighboring bonds in regioisomeric substitution patterns yields unique and characteristic infrared absorption spectra.

The vapor phase infrared spectra for the twelve 1-*n*-pentyl-2-, 3-, 4-, 5-, 6- and 7-(1- and 2-naphthoyl)-indoles show the bridge position on the indole ring is a dominating influence over the observed carbonyl absorption frequency. Substitution on the pyrrole moiety of the indole ring yields the lowest C=O frequency values for position 2- and 3- giving a narrow range from 1656 to 1654  $\text{cm}^{-1}$ . Carbonyl absorption frequencies are higher when the naphthoyl group is attached to the benzene portion of the indole ring yielding absorption values from 1674 to 1671  $\text{cm}^{-1}$ . Furthermore, the inverse isomers (1-(1- and 2-naphthoyl)-3-*n*-pentylindole) show even higher carbonyl absorption in the 1705  $\text{cm}^{-1}$  range.

Characteristic absorption bands for aromatic ethers in the 1500–1200  $\text{cm}^{-1}$  range provide information concerning the position of the methylenedioxy ring and its relationship to the aminoketone side-chain in designer cathinone derivatives. The 2,3-methylenedioxy substitution pattern shows a characteristic absorption band consisting of a strong singlet centered in the 1450  $\text{cm}^{-1}$  range. However, the 3,4-methylenedioxy substitution pattern shows a doublet absorption pattern in the same region. The equivalent region of the vapor phase infrared spectra provides a significant number of unique absorption bands characteristic for each individual dimethoxypropylvalerone regioisomer as well as the aromatic ring substituted precursor aldehydes and synthetic intermediate ketones.

This presentation will describe the use of GC-IR and GC/MS for the specific identification of individual regioisomeric substances from the synthetic cannabinoids, cathinones and N-BOMe drug categories. This work has established numerous chemical structure correlations with infrared spectral absorption patterns.

#### **GC/IR, Synthetic Drugs, Isomer Identification**



### **B178 The Separation of Fentalogs From Complex and Confounding Mixtures Using Gradient Elution Moving Boundary Electrophoresis (GEMBE)**

*Thomas P. Forbes, PhD\**, National Institute of Standards and Technology, Gaithersburg, MD 20899; *David Ross, PhD*, National Institute of Standards and Technology, Gaithersburg, MD 20899

**Learning Overview:** After attending this presentation, attendees will understand how Gradient Elution Moving Boundary Electrophoresis (GEMBE) can be utilized for the separation and detection of fentanyl and various fentanyl analogues from complex mixtures including heroin and other excipients and cutting agents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing the advantages of GEMBE over traditional electrophoretic separations, as well as demonstrating its capabilities for the separation of fentalogs from complex mixtures. The research will present the technique's potential as an analytical tool for the forensic practitioner.

The analysis of fentanyl and fentanyl analogues from complex mixtures remains a challenging and increasingly frequent endeavor for forensic examiners. While their direct detection and identification is imperative, these species are often found as a low abundance component in a mixture containing other narcotics (e.g., heroin, as well as excipients, adulterants, diluents, and bulking agents). To address these complex mixtures, a range of traditional separations are available, including, capillary electrophoresis (CE), gas chromatography (GC), liquid chromatography (LC), and ion mobility spectrometry (IMS). These separations represent Category B analytical techniques for chemical analysis based on SWGDRUG classifications.

Here, gradient elution moving boundary electrophoresis (GEMBE) was employed and its utility characterized for addressing this increasingly prevalent class of samples. GEMBE is a simple microfluidic separation technique consisting of run buffer and sample reservoirs connected by a relatively short capillary (5 cm) and the application of an electric field. Unlike other electrophoretic separations, GEMBE does not require a defined injection. This simplification readily enables easy multiplexing or array formatting to scale up throughput. Separation in GEMBE is achieved by applying pressure to the run buffer reservoir sufficient to drive buffer through the capillary, holding target analytes in the sample reservoir under the application of an electric field. The applied pressure is then ramped down, allowing analytes to sequentially enter the capillary for detection as their electrophoretic velocity overcomes the pressure driven counter flow. The counterglow nature of GEMBE also enables particulates, fibers, and other problematic components in the sample to be excluded from the microfluidic capillary, reducing sample preparation requirements and capillary fouling. Further, the separation resolution for GEMBE can easily be manipulated through the control of the pressure ramp and applied electric field as opposed to the need for increasing channel length or altering the electroosmotic mobility of the channel, typical of traditional capillary electrophoresis.

This study introduces a preliminary analysis of the capabilities and limitations of GEMBE for the separation of fentanyl and a range of fentanyl analogues from complex mixtures. Parametric optimization and analytical performance metrics will be presented for representative cases using predefined mixtures of chemical standards. Methods for enhancing resolution, potential quantitative capabilities, and limits of detection will be discussed.

#### **Fentanyl, Electrophoretic Separation, Complex Mixtures**



### **B179 Gas Chromatography (GC) With Tandem Cold Electron Ionization/Mass Spectrometric (Cold EI/MS) Detection and Vacuum Ultraviolet (VUV) Detection for the Comprehensive Analysis of Fentanyl Analogs**

*Sydney R. Buchalter, BS\*, Washington, DC 20037; Ioan Marginean, PhD, The George Washington University, Washington, DC 20007; Ira S. Lurie, PhD, The George Washington University, Washington, DC 20007*

**Learning Overview:** After attending this presentation, attendees will understand unique benefits of gas chromatography (GC) coupled with cold electron ionization mass spectrometric (cold EI-MS) and vacuum ultraviolet (VUV) detection as a rapid and reliable analytical technique for the analysis of emerging drugs, specifically fentanyl analogs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by investigating an alternative analytical technique that will greatly modernize analysis. This will allow for more rapid analysis, thus aiming to reduce the backlog challenge which impinges many forensic laboratories. The combination of these two complementary detectors in tandem with the high resolving power of the gas chromatograph allows for higher confidence in sample identification by the presence of retention times, enhanced molecular ions with fragmentation, and complementary VUV detection. Additionally, the research aims to demonstrate the value of ultraviolet detection in the gas phase for the analysis of emerging drugs.

GC with electron ionization (GC/MS) is widely used in examination of emerging drugs. While providing predictable and extensive fragmentation patterns, its hard ionization technique yields little or no molecular ions for certain analytes, leading to uncertainty in identification. Conversely, GC coupled with cold EI/MS is based on cooling the molecules emerging from the GC in supersonic molecular beams. The cool molecules have a higher chance of surviving as molecular ions following ionization. In addition to an enhanced molecular ion, the fragmentation pathways are like those obtained using classical EI, enabling the use of established libraries.

Mass spectra generated from GC/MS with both EI and cold EI ionization cannot reliably discriminate between positional isomers, especially when substitution occurs on the benzene ring. UV spectra generated from GC/VUV can distinguish between positional isomers, including those having identical MS spectra. UV detection in the gas phase probes primarily the electronic transitions found in single bonds ( $\sigma \rightarrow \sigma^*$ ) as well as the electronic transitions of double bonds ( $\pi \rightarrow \pi^*$ ), resulting in discernable spectral patterns. These patterns are related to the individual structure of the compound, which may be quickly and accurately identified by a spectral reference library. The high specificity of each spectra not only allows for positional isomer differentiation but for peak deconvolution as well.

GC with flame ionization detection (FID) is also commonly employed in forensic analysis for both screening and quantitation but lacks specificity due to its inability to determine the components under the peak. Conversely, VUV detection allows for quantitation and identification under the peak using deconvolution capabilities, therefore providing for high specificity.

The present study investigates the efficacy of GC interfaced to both cold EI/MS detection and VUV detection by the means of a flow splitter for the simultaneous qualitative and quantitative analysis of twenty-four fentanyl analogues, including seven sets of positional isomers. For 23 analogues, enhanced molecular ions were obtained which were confirmed by MS/MS experiments. The cold EI/MS spectra exhibited similar MS fragments (other than the molecular ion) to those obtained by EI/MS. All the fentanyl related compounds exhibited unique VUV spectra and were successfully identified by a reference library containing hundreds of entries. The repeatability of the interface was demonstrated with both short term (run to run) and long term (day to day) studies further emphasizing accuracy of identification. Stability of the interface is further exhibited through linearity studies using VUV detection for all fentanyl related compounds over a concentration range of 390 PPB to 200 PPM. Additionally, the quantitation value of the interface is exhibited using simulated samples.

*This project is supported by Award No. 2017-R2-CX-0028 awarded by the National Institute of Justice, Office of Justice Programs, and the United States Department of Justice. The opinions, findings, and conclusions expressed in this presentation are those of the authors and may not necessarily reflect those of The Department of Justice.*

#### **Cold Electron Ionization/MS, Vacuum Ultraviolet, Fentanyl Analogs**



### **B180 The Application of Ultra High-Performance Liquid Chromatography-Time-of-Flight/Mass Spectrometry (UHPLC-TOF/MS) to the Analysis of Phenethylamine Derivatives**

*Georgina R. Nicholls, MS\*, The George Washington University, Washington, DC 20007; Ioan Marginean, PhD, The George Washington University, Washington, DC 20007*

**Learning Overview:** After attending this presentation, attendees will understand the methods of analysis for selected substituted phenethylamines (the 2C-x series and 25x-NBOMe series) based on ultra-high-performance liquid chromatography-time of flight-mass spectrometry (UHPLC-TOF/MS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating UHPLC-TOF/MS as a valuable analysis method for substituted phenethylamine compounds. Separation and identification of phenethylamine positional isomers using optimized conditions could significantly increase sample throughput in a forensic drug testing laboratory.

An ever-growing concern to the public health and safety worldwide is the threat posed by an increase in the popularity of emerging drugs. To evade drug laws, synthetic analogues or “designer drugs” are being created by altering the chemical structure of known illicit compounds to mimic the desired effect. Subsequently, the plethora of new drugs appearing on the drug market are being created at a rate that challenges law enforcement efforts to curb their consumption. Phenethylamines are one of the most broad and diverse classes of drugs. Compounds within the class are composed of a core phenethylamine structure consisting of a phenyl ring joined to an amino group via a two-carbon sidechain. The opportunities for derivatization at the aromatic ring, side chain, or amino group are almost endless. For example, the 2C-x series refers to a group of ring-substituted phenethylamines with a methoxy group at the 2 and 5 positions, and generally a lipophilic substituent at the 4 position. In contrast, the 25x-NBOMe series are N-benzyl derivatives of the corresponding 2C-x series, with the substitution of 2-methoxybenzyl on the amine resulting in an increase in the potency.

Due to the high degree of structural similarities, substituted phenethylamines present a challenge when analyzed using traditional techniques such as gas chromatography-mass spectrometry (GC/MS). While GC/MS is the main technique utilized for general forensic drug screening, the analysis of positional isomers poses an important challenge. The lack of discrimination in the fragmentation pathways, in addition to the MS detection yielding little or no molecular ion, results in heavy reliance being placed on retention time for identification. To increase the confidence in the analysis, UHPLC-TOF-MS may be used as an orthogonal identification technique as it provides pseudo molecular ion information and complementary retention time data.

Derivatives of phenethylamines contain substituents which offer varying degrees of size, polarity, and lipophilicity. However, positional isomers differ only in the location of the substituent on the molecule. Hence, the main analysis problem encountered is the discrimination and subsequent identification between the different positional isomers.

In this study, a total of twenty-five substituted phenethylamines were subjected to analysis via UHPLC-TOF/MS. Four different superficially porous (SPP) 150mm x 2.1mm x 2.7µm columns were studied either in reversed phase chromatographic (RPC) mode, hydrophilic interaction liquid chromatographic (HILIC) mode or both. Three columns (C18, Phenyl-Hexyl, and Dimethylpentafluorophenylpropyl (PFP)) were utilized in RPC mode and two columns (HILIC and PFP) were utilized in HILIC mode. For each column, the mobile phase was optimized by either isocratic or gradient conditions to yield optimal separation. Effectiveness of separation was judged based on the ability to resolve the most compounds with special emphasis on the separation of positional isomers. Of the four columns employed, it was determined that the most efficient column was the C18 in RPC mode, which separated sixteen out of the twenty-five compounds. Furthermore, all positional isomers were resolved at a resolution greater than 1.

All positional isomers were separated, and nineteen out of the twenty-five phenethylamine derivatives were resolved by GC/MS. GC/MS provides a higher resolving power but relies heavily on retention time due to lack of discrimination between mass spectra of isomers. UHPLC-TOF/MS uses electrospray ionization (ESI) which yields a pseudo molecular ion and provides accurate mass information. This is an orthogonal technique which provides complementary data to that produced via GC/MS and increases the confidence in the identification of substituted phenethylamines.

#### **Phenethylamines, UHPLC-TOF/MS, Positional Isomers**





### **B181 The Detection and Differentiation of Controlled Substances by Gas Chromatography-Vacuum Ultraviolet (GC-VUV) and Chemometrics**

*Zackery Roberson, BS\*, Greenwood, IN 46142; John V. Goodpaster, PhD, FIS Program, Indianapolis, IN 46202*

**Learning Overview:** The goal of this presentation is to bring an understanding of gas chromatography-vacuum ultraviolet spectrophotometry (GC-VUV) as applied to traditional controlled substances.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the capabilities of gas chromatography-vacuum ultraviolet (GC-VUV) spectrophotometry to detect and differentiate controlled substances.

The region of the electromagnetic spectrum known as “vacuum ultraviolet” extends below 200nm where the electronic transitions of sigma bonds lie. A new Vacuum Ultraviolet (VUV) spectrophotometer has been made available for coupling to gas chromatographs allowing spectra to be obtained from 125-430nm. All gas phase compounds except the smallest molecule, H<sub>2</sub>, absorb above 125 nm.<sup>1</sup> Thus, the VUV detector should be able to detect any molecule analyzable by gas chromatography. Though virtually any molecule can be detected, the question arises of how differentiable the spectra are. To answer this question, several controlled substances were analyzed.

Phenethylamines are a common drug class including pseudoephedrine and illicit drugs such as methamphetamine. Several of the phenethylamines are difficult to analyze by electron impact mass spectrometry due to the fragmentation giving the same mass to charge ratio fragments at similar ratios. While phenethylamines are generally differentiable by retention time, an extra layer of specificity is preferred. Eight structurally similar phenethylamines were found to be differentiable using their VUV spectra. A calibration curve and limit of detection study was performed for two phenethylamines that indicated a limit of detection around 1 ng on-column and upper limit of linearity around 1 µg on-column using gas chromatography–vacuum ultraviolet spectrophotometry (GC-VUV). The spectral data obtained were analyzed by the multivariate statistical techniques Principal Component Analysis and Discriminant Analysis. The results indicate the ability to differentiate each of the phenethylamines based upon their repeatable VUV spectra.

The most exciting results from the phenethylamines are the ability to differentiate the VUV spectra of the diastereomers ephedrine and pseudoephedrine. While the two compounds give very similar spectra, chemometric analyses indicate the ability to correctly associate the spectra with the corresponding molecule. It should be noted that the spectra are similar enough that the 95% confidence interval around the discriminant analysis results overlap slightly. The control software for the VUV detector was also able to distinguish the two with some degree of reliability based on the produced “Match coefficient.” The ability of VUV to distinguish diastereomers also lends itself to the diastereomers of cocaine.

GC-VUV of other drugs such as heroin,  $\gamma$ -butyrolactone, and 3,4-Methyl enedioxy methamphetamine (MDMA) provides results of similar promise for forensic chemistry. Several de-identified seized drugs “street” samples were analyzed to show “real world” performance. It should be noted that VUV has difficulties differentiating small alkanes and should be considered as complimentary to GC/MS rather than a replacement. Overall, GC-VUV continues to show promise for future use in forensic drug analyses as a technique complimentary to GC/MS.

*This work was funded by the National Institute of Justice under grant number 2017-R2-CX-0018.*

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#### **Vacuum Ultraviolet Detection, Phenethylamines, GC-VUV**



**B182 WITHDRAWN**



### **B183 Forensic Science Education: Future-Proof or Flawed Paradigm?**

*Scott Chadwick, PhD\**, University of Technology Sydney, Broadway, NSW 2007, AUSTRALIA; *Claude Roux, PhD*, University of Technology Sydney, Broadway, NSW 2007, AUSTRALIA

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**Learning Overview:** The goal of this presentation is to discuss and highlight the changes in forensic science and the need for education providers to reflect that change in their programs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with: (1) a review of forensic science programs currently offered internationally, (2) an overview of the necessary skills required for future forensic scientists, and (3) a discussion on the challenges facing forensic science as an academic discipline.

A review of forensic science degree programs around the world and industry feedback have shown that in education, forensic science traditionally comes second after core scientific disciplines (such as chemistry or biology); it is not considered as a core discipline on its own. Education providers have designed tertiary courses to produce discipline specific graduates (chemists, biologists, etc.) who are able to give an application of this discipline to forensic science. This has led to the current silos and prevents the development of a unified culture of 'Forensic Science.'

With the advent of the 'smart society' leading to the extraordinary developments of digital forensic science and the changes in operational laboratory practices, there is growing indication that this model presents serious issues and no longer reflects the current state of forensic science. There is a real risk to generate graduates who will not be equipped to face tomorrow's challenges. Part of the problem is that these programs tend to focus on tools rather than problems and rarely consider the complex interpretation, problem solving, critical thinking, and evaluative skills required to be an effective forensic scientist. While the development of laboratory-based skills is important, it is often at the expense of the bigger forensic picture—the value of the trace in court proceedings, in investigative and intelligence processes. This is becoming more of an issue as technological advances are rapidly changing and impact on forensic science workflows. If we focus on the tools, education cannot keep up in this dynamic dimension; the focus needs to be on building better Forensic Scientists.

The presentation will outline the current state and future challenges forensic science education providers face and the need for continued improvement and renewal to ensure the needs of industry, graduates and the discipline of forensic science are met.

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#### **Higher Education, Curriculum, Graduate Outcomes**



### **B184 The Use of a Database Feature in the TrueAllele® Casework System to Cross-Reference DNA Cases**

*Jeffrey Oblock, BS, Cuyahoga County Regional Forensic Science Lab, Cleveland, OH 44106; Nasir A. Butt, PhD\*, Cuyahoga County MEO, Cleveland, OH 44106*

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**Learning Overview:** After attending this presentation, attendees will understand the importance of cross-comparing cases to link crimes and perpetrators.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how TrueAllele® Technology's database feature can assist in the cross comparison of cases to show how the same perpetrators commit crimes using the same modus operandi across various jurisdictions.

Forensic DNA laboratories are very often tasked with solving an abundance of property crimes, including auto thefts. Most of these thefts are believed to be committed by the same perpetrator or group of perpetrators using the same modus operandi. Because of the sheer number of these types of cases and the fact that DNA evidence from these types of crimes generally result in complex DNA mixture data, crime labs are ill-equipped with being able to link various crimes to one another. TrueAllele® Technology's Databasing feature can alleviate these challenges allowing the laboratory to successfully link cases to one another and to perpetrators.

During the summer and fall of 2015, a large group of juveniles terrorized the Cleveland area by stealing vehicles on almost a nightly basis. Multiple law enforcement agencies submitted evidence from the recovered vehicles in the hopes of identifying suspects. A total of 37 suspect reference samples were submitted as part of this investigation for comparison to over 100 evidence samples from upwards of 30 cases. Most of the evidence samples submitted were swabs from various areas from the recovered vehicles, such as, steering wheel, gear shift, and door handles. Many of these swabs resulted in complex DNA mixtures. Profiles were interpreted manually following in-house protocols as well as sent to Cybergenetics for upload into the TrueAllele® system.

After the profiles were interpreted using the automated TrueAllele® software, comparisons were made between evidence-to-evidence profiles to link cases together as well as evidence-to-suspect references to link suspects to multiple cases. Approximately half of the suspect references submitted were linked with a positive match score to at least one auto theft case and in one instance, a suspect was linked in eight cases. In addition, complex profiles that were deemed inconclusive through manual interpretation were linked to suspects using automated TrueAllele® interpretation.

TrueAllele® Technology's Databasing feature was a necessary and invaluable tool used to link multiple auto theft cases and suspects that would have been otherwise impossible using manual methods.

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**TrueAllele® Technology, Databasing, DNA**



### B185 Genotype Information Criteria for Forensic DNA Databases

*John Donahue, MA\*, Beaufort County Sheriff's Office, Beaufort, SC 29906; Mark W. Perlin, PhD, MD, Cybergeneics, Pittsburgh, PA 15213*

**Learning Overview:** The goal of this presentation is to help the attendee better understand effective probabilistic genotyping for DNA database searches.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that an automated probabilistic method for searching DNA databases can use more DNA profile information to increase the number of solved cases with minimal human review effort.

In Bayesian statistics, Kullback-Leibler (KL) divergence is a measure of data information gain from prior probability to posterior probability. Bayesian methods can be applied to forensic DNA mixtures. The KL then estimates the match information present in a contributor's probabilistic genotype. Thus, KL can be used to predict the match statistic when a reference sample is not available.

The Combined DNA Index System (CODIS) is a network of DNA databases shared by law enforcement agencies across the United States. It is primarily used for comparing DNA profiles—crime scene evidence with convicted offenders or arrestees. The CODIS software searches for matches by directly comparing the alleles of two profiles. Depending upon the search criteria, a one-allele profile difference may cause a mismatch that precludes match reporting. In practice, few mismatches occur when comparing highly certain genotypes, such as those derived from a single source evidence sample or an obvious major contributor to a DNA mixture.

However, direct allelic comparison can create challenges when evaluating more complex DNA mixtures. These typically have more genotype uncertainty than single source samples. A direct comparison search of a DNA mixture having several alleles at multiple loci can produce many adventitious candidate matches.

To reduce such false positive results, the National DNA Index System (NDIS) Operational Procedures require that forensic mixtures submitted to NDIS contain at least eight of the original thirteen CODIS core loci, with a maximum of four alleles at any locus. In addition, the mixture must have a match rarity estimate no greater than one in ten million, calculated at moderate stringency for the original CODIS core loci. These restrictions do help reduce adventitious matches. But they also prevent the upload of mixtures that fail eligibility criteria, even if they contain considerable match information. It is therefore likely that current NDIS eligibility criteria prevent the identification of true matches to complex DNA mixtures, which would leave some crimes unsolved.

This presentation describes the database search methods employed by a local laboratory that has a TrueAllele® computer system for developing probabilistic genotypes from complex DNA mixtures. By calculating the KL of a probabilistic genotype, the TrueAllele® computer measures potential match information across all the data, not just at a selected locus subset. The lab uses KL match prediction to assess inferred genotypes for database search suitability and help determine alleles for upload. After a database search returns potential matches, the computer automatically calculates match statistics between the retrieved references and the original inferred mixture evidence.

Direct CODIS allele comparison can return multiple reference genotypes. However, the laboratory's automated TrueAllele® comparison reduces human review time to a few minutes. Moreover, the process provides objective and unbiased match evaluation. This advanced DNA database computing approach has enabled the upload of previously unsearchable profiles to the State DNA Index System (SDIS) and has solved previously unsolvable criminal cases. The results suggest that NDIS might achieve increased database success through KL-directed probabilistic genotyping search.

#### **CODIS, Probabilistic Genotyping, Match Estimation**



### **B186 The First Five Exonerations Using TrueAllele® Statistical Software: How Labs Can Review and Correct Old Cases**

*Greg Hampikian, PhD\*, Boise State University, Boise, ID 83725-1515; Mark W. Perlin, PhD, MD\*, Cybergenetics, Pittsburgh, PA 15213*

**Learning Overview:** The goal of this presentation is to show that reexamination of old DNA samples (or data) with TrueAllele® can produce conclusions in formerly inconclusive cases and can correct interpretation errors in complex mixture analysis. DNA casework that involves complex mixtures, or "below-threshold" alleles can result in analyst misinterpretation or inconclusive results, which can be corrected using fully continuous probabilistic software. After attending this presentation, attendees will know how TrueAllele® software was used to correct five wrongful convictions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that inconclusive (or disputed) DNA results can be easily reexamined without new wet-lab work using TrueAllele® software analysis. Attendees will see that this software has provided exculpatory evidence in 5 exonerations. Some of these exonerations involve DNA that was previously assessed by manual methods with inconclusive results. The forensic community will be shown how this software can review any STR case where the electronic data is available. In one case presented, the community will see how new DNA evidence freed two men after 23 years of a life sentence and identified a new suspect.

TrueAllele® software was used to analyze STR electronic data from crime scene samples in postconviction claims of innocence. This presentation describes the first 5 exonerations using this software and suggest improvements in crime lab processing that can help prevent and correct wrongful convictions.

It has been established by several studies that complex DNA mixtures and low template samples can result in conflicting interpretations and inconclusive results.<sup>1,2</sup> These problems can result in wrongful convictions. Recently developed statistical approaches have produced several software packages to analyze complex DNA mixture analysis and low template DNA analysis. These software approaches are usually combined with traditional manual review.

This study reviews the use of TrueAllele® software by Cybergenetics to examine postconviction claims of innocence. This is a commercial product that uses Marco chain Monte Carlo based, fully continuous probabilistic genotyping. The presentation will cover 5 exonerations that used TrueAllele® analysis. These cases represent DNA mixtures and low template DNA samples, including cases where traditional methods were inconclusive. Reevaluation of electronic data from traditional STR testing was performed by Cybergenetics using TrueAllele® and was corroborated by manual analyst review at Boise State University.

The results demonstrate that TrueAllele® can be used to examine contemporary and old cases—even cases where only electronic STR data remains and no new DNA testing can be performed. In one case, two men were freed after 23 years in prison on dual life sentences for murder. Freddie Lawrence and Paul Jenkins were freed in Montana in 2018. In that exoneration, TrueAllele® analysis (corroborated by the state crime laboratory) produced a full STR profile that matched a new suspect in CODIS. That person has now been named a suspect in the murder.

*The cases described are from a partnership of the Boise State University laboratory of one author (GH), the Montana Innocence Project, the Georgia Innocence Project, the Illinois Innocence Project, and the New Mexico Innocence Project.*

*This work was supported in part by Grant No. (2016-DY-BX-0006) awarded by the Bureau of Justice Assistance. The Bureau of Justice Assistance is a component of the U.S. Department of Justice's Office of Justice Programs, which also includes the Bureau of Justice Statistics, the National Institute of Justice, the Office of Juvenile Justice and Delinquency Prevention, the Office for Victims of Crime, and the SMART Office. Points of view or opinions in this document are those of the author and do not necessarily represent the official position or policies of the U.S. Department of Justice.*

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#### **TrueAllele®, Exoneration, Probabilistic Genotyping**



**B187 WITHDRAWN**

### **B188 The Introduction of Massively Parallel Sequencing (MPS) of Mitochondrial DNA (mtDNA) Into Forensic Laboratory Practice in Croatia**

*Marina Korolija, PhD\*, Zagreb, MD, CROATIA; Viktorija Sukser, MS, Forensic Science Centre "Ivan Vucetic", Zagreb 10000, CROATIA; Sara Rožic, MS, Forensic Science Centre "Ivan Vucetic", Zagreb, CROATIA; Lucija Barbaric, PhD, Forensic Science Centre "Ivan Vucetic", Zagreb 10000, CROATIA*

**Learning Overview:** After attending this presentation, attendees will be acquainted with the challenges of introducing advanced technology into newly established forensic research laboratories. Attendees will learn about some basic principles of massively parallel mtDNA sequencing, data analysis, and actual relevance of mtDNA results in a forensic investigation.

**Impact on the Forensic Science Community:** Given that mtDNA targeted MPS results are globally still scarce, this presentation will impact the forensic science community by contributing substantially to forensic genetics and other scientific fields, such as molecular anthropology and medicine, by extensive and high-quality analysis performed in Croatian population.

Massively parallel sequencing (MPS) has enabled faster, deeper, and wider than ever insight into miracles carried by genomes of living creatures. Sometimes these organisms take part in cases of forensic interest, leaving genetic material as a trace of their physical presence. DNA sequences of complete human mitochondrial genomes, although of great forensic value, were difficult to access until the advent of MPS technology. Time has finally come to dive deeper into the fine structure of human mtDNA phylogenetic tree and a phenomenon of heteroplasmy, thus pushing the limits of current mtDNA forensic applicability. The authors therefore established, optimized, and validated sequencing of mtDNA genome from reference samples, with an initial goal to establish Croatian population database. Extensive database of 299 samples will serve for haplotype comparison and evaluation in future forensic casework. In that context, present efforts strive towards the implementation of MPS mtDNA analysis into routine practice.

Long-range PCR-based approach was chosen for mtDNA enrichment in samples of total genomic extracts from buccal swabs. The whole circular, 16 569 bp long mtDNA molecule was amplified by two sets of primers that overlap in the non-coding, control region (CR). Sequencing library preparation was performed by fragmentation of long PCR products, followed by the addition of oligonucleotides for binding sequencing primers to library fragments, binding fragments to a flow-cell and barcoding them (NexteraXT, Illumina). Sequencing was performed on Illumina MiSeq platform by multiplexing 48 samples per sequencing run, using Illumina MiSeq sequencing chemistry (Reagent kit v2, 300 cycles) and standard flow-cell. Data analysis was performed by both Illumina BaseSpace mtDNA applications, and an in-house bioinformatics pipeline, which is based on experimental and sequencing error modelling for the detection of minor alleles in samples with heteroplasmy. The latter approach is still in its developmental phase. Haplogroups were assigned to samples by importing variants (differences from rCRS) into HaploGrep2 (v2.1.0), a web application based on comprehensive human mtDNA phylogenetic tree (PhyloTree, Build 17).

Average coverage per sample across eight sequencing runs amounted  $6103 \pm 1111$ , which is well above the predicted value of 4633 for the sequencing chemistry used. Macro-haplogroup distribution in Croatian population is as follows: 36% H; 23% U; 8% J and T, each; 6% HV and K, each; 4% V; 2% N1, I and W, each; 1% R0, X and D, each; 0.3% A. Total of 185 haplotypes are assigned to 299 analyzed samples. However, in cases when several samples share the same haplotype, the necessity of a more refined haplogroup classification within PhyloTree is evident. Regarding point heteroplasmies (PHs), as much as 27.8 % of samples exhibit minor alleles at frequency  $\geq 10\%$  (approximate detection limit of Sanger sequencing). In 4.3% of samples, there are two or more PHs present, and the most common PH (16093Y) appears in 3.3% of samples. Detected PHs are mostly transitions, distributed compactly in approximately 1.1 kb long CR (30 of them) and more sparsely distributed throughout approximately 15.4 kb of the mtDNA coding region. Low-level (<10%) variant detection is still being optimized.

In conclusion, with accurate information on sequencing library in terms of quantity and quality, it is possible to outperform manufacturer-specified output of the flow-cell and sequencing chemistry, without compromising the quality of the run. Apart from cost-effectiveness, it improves sensitivity of minor allele frequency detection. Distribution of macro-haplogroups in Croatia matches that found in Europe. Haplotypes determined in this study will enable further branching of global mtDNA phylogenetic tree, especially in the most common European haplogroup H. Optimized protocol for whole mitogenome sequencing and heteroplasmy detection, along with Croatian mtDNA database, is going to be used in routine forensic practice in Croatia, primarily in human identification, when standard Sanger sequencing of mtDNA CR does not provide sufficient power of discrimination.

#### **Mitochondrial DNA, Massively Parallel Sequencing, mtDNA Forensic Database**





### **B189 A Reproducible Failure of One Base Pair Resolution in Casework Samples on the 3500xL Genetic Analyzer**

*Rachel Maragliano\**, Huntington, WV 25705; *Jason M. Chute, MSFS, Marshall University Forensic Science Center, Huntington, WV 25701*; *Kelly Beatty, MSFS, Marshall University Forensic Science Center, Huntington, WV 25701*

**Learning Overview:** After attending this presentation, attendees will understand the forensic DNA workflow, the importance of the ability for instrumentation to accurately resolve DNA mixtures, and a recurring failure to resolve DNA mixtures with alleles one base pair apart.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by confirming a one base pair resolution failure and present some observations that can lead to fixing this issue.

Many items of evidence submitted for forensic DNA testing are mixtures of more than one individual. Therefore, it is critical that the instrumentation and chemistries used can correctly resolve all the alleles from all contributors. Recently, laboratories have been noticing that a mixture consisting of a homozygote as the major contributor and a heterozygote as the minor contributor at a locus where two of the alleles, the homozygote allele and one allele from the heterozygote within one base pair of each other are not properly resolving. At initial glance it appears to be a non-Gaussian peak caused by artifacts of the DNA process. However, standards require precision studies exhibiting one base pair resolution, so if it is not occurring that is cause for alarm. This study looked to replicate this resolution failure and to provide some insight as to why this issue is occurring.

The first study looked to see how well resolution occurred in the position of the minus A artifact. Samples were obtained from two females who at D12S391 are 18,18 (female A) and 17.3,20 (female B). Extracts were quantified, normalized, and mixed together at ratios of 1:1, 2:1, 4:1, 6:1, 8:1, 10:1, and 16:1, with female A as the major contributor. The mixtures were then amplified with a GlobalFiler™ PCR Amplification Kit at 1 ng and 3 ng, run on a 3500xL Genetic Analyzer, and analyzed with GeneMapper® *ID-X* v1.5.

The second study looked at resolution where the homozygote microvariant is the major contributor and a heterozygote is the minor contributor. This was to see the resolution on the trailing side of the homozygote peak. Samples were obtained from a female and a male who at TH01 are 9.3,9.3 A (female C) and 6,10 (male A). Extracts were quantified, normalized, mixed together at ratios of 1:1, 2:1, 4:1, 6:1, 8:1, 10:1, and 16:1. The mixtures were then re-quantified in triplicate to obtain actual mixture ratio values. The mixtures were then amplified in triplicate with a Globalfiler™ PCR Amplification Kit at 0.5 ng and 1.5 ng loads and run on a 3500xL Genetic Analyzer. The mixtures, apart from the 16:1, were also amplified at 0.5 ng and 1.5 ng loads with PowerPlex® Fusion 6C System and run on the same instrument, however, not in triplicate.

In the first study, the mixtures amplified with a 3 ng/μL load resolved better than those with a 1 ng/μL load. Six out of the seven mixtures with a 3 μL load resolved the 17.3 allele, while three of the six mixtures with a 1 ng/μL load resolved. Analysis of the peak height ratios show that the minus A artifact is contributing to the peak height, causing the peak to be resolved.

In the second study, only one of the 4:1 mixture replicates and the 1:1 and 2:1 mixtures amplified with GlobalFiler™ resolved. However, the 1:1, 2:1, 4:1, and 6:1 mixtures amplified with PowerPlex® Fusion 6C resolved the 10 allele at both amplification loads and the 8:1 at a 1.5ng load. This confirms that without the minus A artifact, alleles within one base pair of each other are not resolving correctly.

While there is no definite answer yet as to what is causing the resolution failure, it is to note that PowerPlex® Fusion 6C did resolve the mixtures better at lower mixture ratios than Globalfiler™. This could possibly be due to the dynamic range of the 3500xL increasing to greater than 10,000 RFU, while the analytical threshold did not increase proportionally. Future studies look to investigate this cause further, such as using a different polymer, running on a different capillary electrophoresis instrument, adjusting run voltage, and amplifying with an Investigator 24plex QS Kit.

#### **DNA, Mixtures, Resolution**



### B190 Bias in Quantitative Polymerase Chain Reaction (qPCR): Does It Matter for Forensic Applications?

Erica L. Romsos, MFS\*, National Institute of Standards and Technology, Gaithersburg, MD 20899; Steven P. Lund, PhD, National Institute of Standards and Technology, Gaithersburg, MD 20899; Peter M. Vallone, PhD, National Institute of Standards and Technology, Gaithersburg, MD 20899-8314

**Learning Overview:** After attending this presentation, attendees will understand the different sources of bias in quantitative PCR (qPCR) and the impact on downstream Short Tandem Repeat (STR) genotyping processes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by bringing attention to the differences in quantitation standards as well as differences in commercial quantitation kits. It will aid the forensic community in better understanding bias and its effect on downstream processes such as STR genotyping.

Commercial STR assays used by the forensic human identity community require tight control of the amount of DNA template amplified by PCR. This requires the ability to accurately measure the concentration of human genomic DNA in a casework sample extract prior to PCR amplification. Commercially available qPCR kits are routinely relied upon to determine the concentration of casework extracts; however, these kits rely upon commercial DNA standards for relative quantitation estimates. qPCR measurements do not provide the absolute concentration of a DNA extract but rather the relative concentration compared to the standard curve. Variation in these quantitative measurements may lead a laboratory to possibly overestimate or underestimate the concentration of an unknown extract and that may lead to incorrect dilution into the PCR workflow.

Variation that may lead to bias in qPCR results originate from a variety of other sources such as, but not limited to, pipette errors, commercial kit targets, accessibility of the amplification target within the human genome, and variation in the concentration of commercial DNA standards present within qPCR kits.<sup>1</sup> Additionally, it has been shown that the source of commercial DNA standards (i.e., blood versus cell lines) can impact the reliable quantification of DNA extracts and prompted the need for an international standard reference DNA material.<sup>2,3</sup>

To investigate the sources of bias, a set of 80 human blood samples (spanning across three U.S. populations) were evaluated with three commercial qPCR kits. This set of samples allows for sample bias to be investigated along with commercial qPCR kit bias. Standard curves were generated with the commercial DNA standard provided within the qPCR kit and SRM 2372a to investigate the effect on the quantification on unknown extracts.<sup>4</sup>

To examine the impact (if any) of the downstream PCR process, STR genotyping was conducted on a subset of the samples. Additionally, droplet digital PCR (ddPCR) was used to determine the quantity of the unknown extracts examined. Digital PCR allows for absolute quantitation of an extract without the need of an external calibrant. Recently, the National Institute of Standards and Technology (NIST) developed ten ddPCR assays that span 8 chromosomes within the human genome for the certification of SRM 2372a.<sup>5</sup>

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#### SRM 2372a, Quantitative PCR, qPCR



### **B191 The Development of the National Institute of Standards and Technology (NIST) Polymerase Chain Reaction (PCR) -Based DNA Profiling Standard (Standard Reference Material 2391d): Where Are We Now?**

*Becky Steffen, MS\*, National Institute of Standards and Technology, Gaithersburg, MD 20899; Erica L. Romsos, MFS, National Institute of Standards and Technology, Gaithersburg, MD 20899; Kevin Kiesler, MS, Gaithersburg, MD 20899; Katherine B. Gettings, PhD, National Institute of Standards and Technology, Gaithersburg, MD 20899; Lisa Borsuk, MS, National Institute of Standards and Technology, Gaithersburg, MD 20899; Peter M. Vallone, PhD, National Institute of Standards and Technology, Gaithersburg, MD 20899-8314*

**Learning Overview:** After attending this presentation, attendees will understand the comprehensive nature of the data associated with the candidate NIST Standard Reference Material 2391d: PCR-Based DNA Profiling Standard and how this will aid the forensic community.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the benefits of such an extensively characterized standard reference material with all commercially available forensic DNA markers at the time of certification. The certified reference material enables the calibration of the typing process for Short Tandem Repeat (STR) and other sequence-based markers for human identity testing.

There have been multiple iterations of the NIST Standard Reference Material (SRM) 2391: PCR-Based DNA Profiling Standard since 1995 when it was first produced. The authors are currently working on SRM 2391d, the fifth installment in a long line of predecessors. The DNA forensic community has progressed greatly in the 23 years since the first release of SRM 2391, which was certified initially for variable number tandem repeat (VNTR) and dot-blot hybridization markers and was later updated with autosomal short tandem repeat (STR) markers. The subsequent two iterations, 2391a and 2391b, were certified for only autosomal STR markers, but it wasn't until the currently available SRM 2391c was released in 2011 and later updated in May of 2018 that other forensically-relevant markers were added to the Certificate of Analysis (COA), including Y-STR markers, X-STR markers, insertion/deletions (indels), and single nucleotide polymorphism (SNPs).<sup>1</sup> The goal for SRM 2391d is to provide a highly characterized set of genomic samples for all commercially available forensic DNA markers at the time of certification, comprised of all the markers previously included in SRM 2391c as well as mitochondrial DNA (mtDNA) whole genome sequences, insertion/null alleles (INNULS), and microhaplotypes (pending availability). The range of certified, reference and information values that will be associated with this SRM, how these values are assigned, as well as the various methods used to obtain these values including capillary electrophoresis (CE) and next generation sequencing (NGS) will be discussed.<sup>2</sup>

The original plan for the development of SRM 2391d has slightly changed throughout the production and certification process. Therefore, these changes will be highlighted and explained in this presentation.

Finally, the question, "Where are we now?" will be answered. The currently available SRM 2391c is expected to be depleted in April 2019 based on reported sales. This SRM is used to address the U.S. Federal Bureau of Investigation's (FBI) Quality Assurance Standards (QAS) for laboratories conducting forensic DNA testing that were published in 2000 and updated in 2011 (Sect. 9.5.5): which currently states, "The laboratory shall check its DNA procedures annually or whenever substantial changes are made to a procedure against an appropriate and available NIST standard reference material or standard traceable to a NIST standard."<sup>3</sup> This is one reason of many that there must not be a lapse in availability for this SRM. The plans moving forward for future updates will also be outlined.

#### **Reference(s):**

1. SRM 2391c:PCR-Based DNA Profiling Standard Certificate of Analysis (2015). Available online at <https://www-s.nist.gov/srmors/certificates/2391c.pdf>. Accessed July 27, 2018
2. Thompson, A.; Taylor, B.N.; Guide for the Use of the International System of Units (SI); *NIST Special Publication 811*; U.S. Government Printing Office: Washington, DC (2008); available at <https://www.nist.gov/physical-measurement-laboratory/special-publication-811>. Accessed July 27, 2018
3. Quality Assurance Standards (QAS) for Forensic DNA Laboratories (2011). Available online at <https://www.fbi.gov/file-repository/quality-assurance-standards-for-dna-databasing-laboratories.pdf/view>. Accessed July 27, 2018

#### **Forensic DNA, Standard Reference Material, STR Markers**



### **B192 Increasing Productivity Through Process Management: The Experience of the Brazilian Federal Police DNA Laboratory**

*Ronaldo C. da Silva, Jr., PhD\*, Brazilian Federal Police, Brasília, Distrito Federal, BRAZIL; Levy Heleno Fassio, Brazilian Federal Police, Brasília, BRAZIL; Jorge Freitas, PhD, Brazilian Federal Police, Brasília 70610200, BRAZIL; Renato T. Ferreira de Paranaíba, BA, Brazilian Federal Police, Brasília, Distrito Federal 70610200, BRAZIL; Renata S. Paiva, BS, Brazilian Federal Police, Brasília, BRAZIL; Ana Paula V. Castro, MSc, Brazilian Federal Police, Brasília, BRAZIL; Carlos B. Carvalho, PhD, Brazilian Federal Police, Brasília, Distrito Federal 70275110, BRAZIL; Bruno R. Trindade, MSc, Brazilian Federal Police, Brasília, BRAZIL; Jeferson L. Badaraco, MSc, Brazilian Federal Police, Brasília, BRAZIL; Aline C. Minervino, MSc, Brazilian Federal Police, Brasília, BRAZIL; Carlos Eduardo M. Medeiros, BS, Brazilian Federal Police, Brasília, BRAZIL; Helio Lima, PhD, Park WAV, Brasília, BRAZIL; Sergio M. Aguiar, MSc, Brazilian Federal Police, Brasília, BRAZIL*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### B193 A Characterization of Sexual Assault Lubricants: Lubricant Database Use in an Operational Setting

Brooke R. Baumgarten, BS\*, Winter Park, FL 32792; Caterina Vadell-Orsini, National Center for Forensic Science, Orlando, FL 32816; Mark Maric, PhD, Orlando, FL 32816; Candice Bridge, PhD, National Center for Forensic Science, Orlando, FL 32816

**Learning Overview:** After attending this presentation, attendees will understand the value of the classification of lubricants as a novel forensic technique, as well as the value of the National Center for Forensic Science (NCFS) Lubricant Database for the analysis of sexual assault trace evidence, in contrast to current methods of solely identifying the presence of lubricant. This database will provide analytical results from Direct Analysis in Real Time-Time-Of-Flight Mass Spectrometry (DART-TOFMS), Gas Chromatography/Mass Spectrometry (GC/MS), and Fourier-Transform Infrared Spectroscopy (FT-IR).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by aiding in the classification of unknown sexual lubricant samples based on components that are indicative of a certain type or class. The results will be provided in a sexual lubricants database made available to the forensic community, thus providing investigative leads by facilitating a way to classify unknown samples.

Unfortunately, sexual assaults are a reality in today's society. The increased use of condoms may be one reason why there is a potential reduction of finding DNA evidence in sexual assault cases<sup>1</sup> This dilemma has led to research in other trace evidence such as sexual lubricants. In the absence of DNA, the analysis of trace lubricant residue may aid in connecting the suspect to the victim and/or crime scene.<sup>2</sup> Previous research at NCFS has shown the capability of identifying discernable groups within lubricant manufacturing types, as well as the ability to separate lubricants from personal hygiene products.<sup>3-5</sup> The classification and characterization of lubricants is a relatively new approach in analyzing trace lubricant residue in an operational setting. In this study, 115 samples from different sexual lubricant manufacturing types were analyzed: water-based, silicone-based, oil-based, organic/edible lubricants, and others (e.g., personal hygiene products). Based on manufacturing information, the tested lubricant samples may be sub-classified as regular, sensation, flavored, spermicidal, and anesthetics. However, if analyzing an unknown sample, the classification must be based on the components identified in the sample.

This research project developed DART-TOFMS, GC/MS, and FT-IR methods and protocols that can be used to analyze samples and identify components in sexual lubricants. The resulting spectra from each instrument was used to create a classification scheme, all of which can be found in the NCFS Lubricant Database and can be used to classify unknown lubricant samples and generate investigative leads. Neat lubricants as well as hexane and methanol extracts were analyzed using DART-TOFMS in both positive and negative ionization modes with a helium carrier gas at 350°C and an orifice 1 voltage of 20V. Neat lubricants and extracts were analyzed in triplicate via the DART-TOFMS as well as FT-IR, and only the extracts were analyzed by GC/MS in triplicate. Multivariate statistical techniques were used to create a classification scheme for the lubricants from the DART-TOFMS, GC/MS, and FT-IR combined results. The classes were differentiated based on unique components specific to that class and the relative intensity of commonly observed components. Additionally, components that aid in designating the sub-class of the lubricant will be presented. The classification scheme developed from this study and provided along with the database will allow analysts to classify unknown lubricants and lubricant residues found at a crime scene using one of the aforementioned instrumentation. A brief overview of this database, classification scheme, and its applicability will be presented.

#### Reference(s):

1. O'Neal, E. N.; Decker, S. H.; Spohn, C.; Tellis, K., Condom use during sexual assault. *Journal of Forensic and Legal Medicine* 2013, 20 (6), 605-609.
2. Musah, R. A.; Cody, R. B.; Dane, A. J.; Vuong, A. L.; Shepard, J. R. E., Direct analysis in real time mass spectrometry for analysis of sexual assault evidence. *Rapid Communications in Mass Spectrometry* 2012, 26 (9), 1039-1046.
3. Maric, M.; Bridge, C., Characterizing and classifying water-based lubricants using direct analysis in real time@-time of flight mass spectrometry. *Forensic Science International* 2016, 266, 73-79.
4. Moustafa, Y.; Bridge, C. M., Distinguishing sexual lubricants from personal hygiene products for sexual assault cases. *Forensic Chemistry* 2017, 5, 58-71.
5. Baumgarten, B.; Marić, M.; Harvey, L.; Bridge, C. M., Preliminary classification scheme of silicone-based lubricants using DART-TOFMS. *Forensic Chemistry* 2018, 8, 28-39.

#### Sexual Lubricants, Database, Classification



### B194 Hair as a Trap for Evidence and a Tool for the Forensic Investigation

*Giulia Caccia, BSc, LABANOF, Università degli Studi di Milano, Milan, ITALY; Annalisa Cappella, PhD, LABANOF, Sezione di Medicina Legale, Milano 20133, ITALY; Alberto Amadasi, MD\*, Università di Bologna, Bologna 40126, ITALY; Debora Mazzarelli, BS, LABANOF, Sezione di Medicina Legale, Milano 20133, ITALY; Agostino Rizzi, BSc, CNR, Milan; Cristina Cattaneo, PhD, LABANOF, Sezione di Medicina Legale, Milano 20133, ITALY*

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**Learning Overview:** After attending this presentation, attendees will understand the importance of a thorough analyses of hair and its potential in providing crucial information within forensic casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the effectiveness of hair as a substrate even in the presence of nuisance factors such as environmental contaminants.

Hair is perhaps the largest and most preservable interface between human body and environment. Hair is frequently preserved along with skeletal remains, but what is the ability of the hair to retain evidences if exposed to environment? And which are the effects of deterioration of an open environment? How long can biological evidence be detected and determined on hair? Given the lack of literature, this pilot study tested the effectiveness of hair as a “trap” of evidence and resource for forensic investigation.

Fifty-seven samples of hair, taken from a single donor, were taken had substrata. One-third of the samples were treated with two biological samples (blood and semen taken from two donors), chosen because of their frequency in forensic contexts. All samples were monitored for a maximum period of three months. Samplings were performed after 24 hours, 48 hours, 1 week, 1 month, 2 months, and 3 months. Each sample was analyzed with the following techniques:

- macroscopic and mild magnification analyses using episcopes microscope
- morphological and chemical analysis using scanning electron microscope Cambridge Stereoscan 360
- analyses with light microscopy and hematoxylin-eosin staining
- Luminol test

**Results on blood:** close environment-bloodstains were macroscopically visible until the end of the trial (3 months), though with a change in color. The basic pool of chemical elements (chloride, sulphur, potassium, sodium, phosphorus) was always recognizable. Luminol provided strong and positive evidence until the end of the trial.

Open environment-blood stains were macroscopically invisible starting to 1 week of trial (Time 3). The basic pool of chemical elements was extremely altered and no longer recognizable over 1 month (Time 4). Luminol gave strong and positive evidence until 1 week, then the intensity decreased.

**Results on semen:** close environment-semen stains were macroscopically visible until the end of the trial (3 months). The basic pool of chemical elements (sodium, chloride, potassium, phosphorus, sulphur) was always recognizable. The additional technique of optical microscopy showed well preserved semen until the end of the trial.

Open environment- semen stains were macroscopically invisible starting to 1 week of trial (Time 3). The basic pool of chemical elements was extremely altered and no longer detectable from 1 week (Time 3). The additional technique of optical microscopy showed detectable sperm until 1 week and sperm still partly visible but severely altered.

The efficacy of hair as evidence was highlighted. Results also showed the influence of environment and time, as blood and semen were detectable until the end of the trial in close environment but not in open space. Regarding blood, Luminol was the most effective technique both in close and in open environment, regardless of contamination and dilution. For what concerned semen, optical microscopy was the most reliable technique even if a most specific coloring could narrow the problems of determinability since some structure may be visible until the end of the trial. The present pilot study showed the effectiveness of hair as a substrate even in the presence of nuisance factor such as environmental contaminants (rain, mineral elements, etc.), further studies on techniques could therefore be taken forward.

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### Forensic Pathology, Hair, Biological Fluids



### **B195 Identifying Paradoxical Undressing During Investigations: A Case Study**

*Adam C. Armstrong, MS\*, U.S. Army Criminal Investigation Division, Fort Bragg, NC 28310*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### B196 Using Drones as a Valid Alternative to Employing Traditional Documentation Systems at Crime Scenes

*Luciano Garofano, PhD\*, President of Accademia Italiana di Scienze Forensi, Parma 43100, ITALY*

**Learning Overview:** After attending this presentation, attendees will understand the possibility of using drones as a valid alternative to employing traditional documentation systems at a crime scene.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the advantages of using drones to preserve crime scene integrity while assuring a thorough photo and video recording.

The documentation of the crime scene is an increasingly decisive activity for reconstructing the dynamics of a crime. The availability of digital tools and specific lighting techniques such as forensic lights have also widened the possibility of identifying and distinguishing latent traces. Decisive, important data can also be obtained using miniaturized 3D laser scanners which offer a further opportunity to obtain precise measurements and extremely interesting models for reconstructive purposes.

However, the problem of contamination remains partially unsolved because taking photos, videos, and the calculations of dimensions and distances forces the operators to move within the crime scene and therefore to contaminate it or change the original conditions, involuntarily, despite anti-contamination procedures even if in compliance with relevant safeguard guidelines.

This is what led the author to think about recent technological progress and how it has provided the availability of instruments such as drones, which can be an extraordinary alternative to the traditional systems which are currently used today, especially when considering that the models that are on the market today are small, easy to maneuver, have high quality cameras, and take excellent photos. Drones are also compatible with dedicated photogrammetric software with which you can build 3D models, maps, and orthomaps with the relative measurements. This is all possible thanks to footage which is taken outside the crime scene.

A wide experiment was conducted through a considerable series of tests with scenarios built ad hoc, varying the environments (indoor/outdoor), lighting conditions, and weather conditions.

The experiment conducted the following tests, using two different drones, the DJI SPARK and the DJI MAVIC AIR manufactured by DJI:

- Outdoor relief with high temperature
- Outdoor relief with low temperature
- Outdoor relief with fog
- Outdoor relief with snow
- Outdoor relief with rain
- Outdoor relief with lots of light
- Outdoor relief with little light
- Relief outdoor with darkness
- Outdoor relief with smoke
- Outdoor relief with medium wind
- Relief outdoors with strong wind
- Inspection inside a well
- Inspection inside a farmhouse
- Search for a fugitive
- Search for a missing person
- Water-based research
- Test in inaccessible areas / woods / without GPS
- High altitude test
- Relief indoors with light
- Embossed indoors
- Indoor relief completing dark
- Switching from windows and doors
- Relief indoors in a large place
- Relief indoors in a tight place
- Relief in the shower/bathtub

The results obtained are exciting and show that drones are a tool that not only can be considered a valid alternative to the systems in use, but they also provide even greater opportunities such as the advantage of a secure safeguard of the crime scene. The presentation will show some of the results that have been obtained through images and videos taken from the experiment performed.

#### **Drones, Crime Scene, Photo And Video Documentation**

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### **B197 Detection of Fentanyl Analogs in Soil Via Paper Spray-Mass Spectrometry**

*Sarah Dowling, BS\*, Whitestown, IN 46075; Trevor Glaros, Aberdeen Proving Ground, MD 21010-5424; Nick Manicke, Indianapolis, IN 46202*

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**Learning Overview:** After attending this presentation, attendees will better understand the use of paper spray-mass spectrometry to detect trace amounts of fentanyl analogs in soil.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community because of its applicability to the ongoing opioid crisis. The analysis of soil samples would be a rapid and effective way to determine the identity and quantity of fentanyl analogs in the environment.

Synthetic opioids are potent narcotic analgesics that are only used legally as a last resort to combat extreme and often terminal pain. Fentanyl analogs are the source of tens of thousands of overdoses across the United States per year. To fuel the opioid epidemic, these analogs are being mass-produced in clandestine laboratories to be sold on the illicit drug market. These clandestine laboratories are a growing environmental concern due to their potential to have a detrimental impact to an ecosystem. There is also a significant forensic interest to be able to identify the opioids being synthesized in clandestine laboratories. Due to their hydrophobicity and large octanol-water coefficients, fentanyl analogs are likely to accumulate if introduced into soil.

In this research, paper spray ionization was utilized in conjunction with a triple quadrupole mass spectrometer and a hybrid quadrupole orbitrap mass spectrometer to detect trace amounts of fentanyl analogs in soil. Paper spray ionization is a fast and easy alternative to typical chromatographic techniques. Soil analysis would typically require advanced clean up procedures before introduction to a chromatography column. However, paper spray eliminates a majority of the sample preparation.

Three soil systems—Richfield clay loam, sassafras sandy loam, and sand, were studied due to their differing pH, organic content, and mineral composition. A mixture of fentanyl analogs was spiked into soil and allowed to interact with the matrix for approximately 24 hours. Two extraction techniques were utilized. A rapid on-cartridge extraction was established for qualitative and semi-quantitative analysis. This technique has the potential to be utilized in the field with a portable mass spectrometer. A modified Quick Easy Cheap Effective Rugged and Safe (QuEChERS) extraction was utilized for quantitative analysis. In this extraction, an acetonitrile organic phase was added to the soil along with salts that forced the analytes into the organic phase.

Analogs were monitored using parallel reaction monitoring on a Q-Exactive for the online extraction method and selected reaction monitoring on a TSQ Vantage for the QuEChERS extraction method and matrix effects study. It was determined that there were no matrix effects caused by the soil. The limits of detection of the QuEChERS extraction method will be discussed.

Fentanyl analogs are an increasing public health concern due to their potency and addictive properties. Due to their increasing prevalence in society, it is more important now than ever to be able to identify and quantify trace amounts of these deadly compounds in the environment.

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### **Fentanyl, Soil, Paper Spray-Mass Spectrometry**



### **B198 Forensic Analysis of the Soil Microbiome: Linking a Piece of Evidence to a Location**

*Jill H. Cocking, MSc\*, Northern Arizona University, Flagstaff, AZ 86011; Greg A. Caporaso, PhD, Northern Arizona University, Flagstaff, AZ 86011; Viacheslav Y. Fofanov, PhD, Northern Arizona University, Flagstaff, AZ 86011; Ryan Turley, Flagstaff Police Department, Flagstaff, AZ 86001; Crystal Y. Hepp, PhD, Northern Arizona University, Flagstaff, AZ 86011*

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**Learning Overview:** The goal of this presentation is to share an interesting case where a soil microbiome (community of microorganisms) was used to assist law enforcement in identifying a probable location visited by a murder suspect.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how soil microbiome analysis, a trending area of biological research, may be able to provide a new investigative tool to law enforcement by associating a piece of evidence with a particular location.

Advancements in DNA sequencing technologies have allowed scientists to learn about the diverse population of microorganisms that inhabit the human body and the world around us. Understanding microbiome diversity has become increasingly important in a wide range of research from human health to bioremediation. In forensics, changes in the microbiome upon mammalian death has already been analyzed in many studies and can be used to accurately estimate the time of death. Different soils harbor their own community of microorganisms that vary based on many factors, including location. This inherent variation may be useful for forensic purposes.

In 2017, the Flagstaff Police Department requested assistance in locating a missing woman. A suspect in the case was in custody and a sock believed to have been worn by this individual was in the possession of the police department. Importantly, police suspected that the corresponding shoe had been lost between the time the woman went missing and the eventual arrest. Police hoped an analysis of the sock might indicate a possible location of the missing woman. During discussions about whether this type of analysis was possible, the body of the missing woman was recovered. Nevertheless, an analysis of the sock was completed to show proof of concept with the hope that the results could be useful for future investigations.

DNA was extracted from the sock and from soil collected at a variety of locations around Arizona. Two methods were used to extract DNA from the sock. The first was to extract DNA from a cutting of the sock itself. The second method was to swab the exterior of the sock with a moistened cotton swab and then extract from the swab. The latter method was found to contain less human-associated bacteria and was therefore determined to be the better method for obtaining the soil bacterial community. A highly variable portion of the 16S gene found in microbes was amplified and sequenced. The resulting 16S sequences were analyzed using Qiime™ 2, a microbiome bioinformatics tool. The microbial community composition from the sock samples were compared to that of the soil collected at locations that the suspect visited. The soil samples from different locations were varied in their microbial composition and abundance, and the microbial community composition of the sock was most like a single location. Therefore, the authors were able to narrow down the options of where the soil on the sock had originated, given a known set of possible locations. The results indicate that a piece of evidence may be linked to a general location and microbial community analysis could be used as an investigative tool for law enforcement.

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**Microbiome, Soil, Qiime™ 2**



### **B199 Redefining Crime Scene Investigation**

*Michelle D. Miranda, PhD, Farmingdale State College, SUNY, Farmingdale, NY 11746; Peter R. De Forest, DCrim\*, Forensic Consultants, Ardsley, NY 10502; Rebecca E. Bucht, PhD, NBI Forensic Laboratory, Vantaa 01301, FINLAND*

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**Learning Overview:** After attending this presentation, attendees should consider and perhaps reevaluate what characterizes appropriate crime scene investigation and a crime scene investigator.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by drawing attention to the shortcomings of the extant role of a crime scene investigator as technician versus the more appropriate role as a scientific crime scene investigator as an educated individual with skills in observation, critical thinking, and reasoning that make for more effective recognition of traces at crime scenes.

The terms commonly used to describe investigative activities at a crime scene are misleading. These terms including collection, recovery, processing, and gathering seem to imply that the important traces that can inform the investigation and provide details about the activities taking place during the commission of the crime are lying about in plain sight ready to be documented, picked up, and packaged for transport to the laboratory.

Unfortunately, this is the dominant conceptualization and it also typifies existing practices with respect to education, training, and fieldwork. The most crucial elements—recognizing traces and making an informed selection of which items and traces to document and collect—does not receive the attention and appreciation it deserves. Such passive, rote practices ignore the complexity of crime scene investigations and the need for scientific reasoning and problem-solving approaches. Because of a scientifically naïve defining and circumscribing of the problem or event being investigated and the consequent selection of potential traces, these practices severely compromise the subsequent laboratory investigation. The *most relevant* traces may go unrecognized and be left at the scene and thus limit the potential contributions of the laboratory.

It must be borne in mind that at the outset of the scene investigation, the specific scene presents a new and unique situation that cannot be approached with a formulaic mindset. No two crime scenes are ever exactly alike. Extensive crime scene experience with past cases is valuable and indispensable, but it must not be relied on to the extent that any prior case is used as a confining template for the investigation of the “latest” one. It is proposed that agencies move away from the technician driven “bagging and tagging” of traces at the scene and move towards more science-based investigative methods. This should be done by assigning these critical responsibilities to experienced scientists properly educated and trained in the scientific method and critical reasoning, and further contextualize the specific aspects of a scene to facilitate the recognition of the possible resultant traces.

In addition to proposing some key knowledge, skills, and abilities that a crime scene scientist should master and in highlighting deficiencies in existing education and training practices, this presentation will demonstrate the potential of unrecognized evidence in the form of cases where the inappropriate handling of traces at the crime scene had significant (negative) effects on the forensic investigation. Considering recent attention to bloodstain pattern analysis, the presentation will consider cases where passive crime scene investigation and lack of applied critical thinking impacted interpretation and relevance of blood traces.

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### **Crime Scene, Scientific Investigation, Scientific Reasoning**



### **B200 Rise of the Machine and the Demise of the Forensic Science Laboratory?**

*Brooke W. Kammrath, PhD\*, University of New Haven, West Haven, CT 06516; David San Pietro, PhD\*, University of New Haven, West Haven, CT 06516; Peter R. De Forest, DCrim\*, Forensic Consultants, Ardsley, NY 10502*

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**Learning Overview:** After attending this presentation, attendees will understand the benefits and potential risks of the uncritical implementation of advanced technologies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by initiating a discussion on the consequences of advances in technology and its effect on forensic science laboratories.

'Rise of the Machine' in this title refers to two emerging technologies that have an impact on the field of forensic science: laboratory automation and field deployable instrumentation. These two technologies can each have profound implications on the effective practice of forensic science in all areas of the physical evidence continuum.

Laboratory automation has already impacted analytical methods in forensic laboratories, improving efficiency and casework flow, thereby decreasing turn-around times. This has enabled an increased time for the more critical front-end issues of informed specimen selection. If this advantage of the role of the scientist in front-end evidence assessment is not appreciated, and this step is omitted, then there is a risk of the misapplication of this technology rendering any perceived advantage doubtful.

Field deployable instrumentation (i.e., rapid DNA analysis, hand-held infrared and Raman spectrometers, portable gas chromatography/mass spectrometers) has provided the potential for on-the-scene analysis by non-scientist investigators. The perceived advantage of these technologies is the analysis of materials with immediate and easy-to-understand results that can be used in real-time investigations. These instruments have utility in the relatively simple and straight-forward analyses of materials such as illicit drugs. However there are concerns with their use in complex crime scene investigations and reconstructions. There are numerous potential dangers with this tempting approach, including evidence destruction, the failure to recognize critical traces, an absence of scientifically informed specimen selection process, and the production of computer algorithm-based unverified conclusions and identifications. There is evidence of this already taking place with respect to scene investigations with connection to the rote use of swabbing in lieu of scientifically-based selection.

This directed discussion with potential audience participation will focus on suggestions for the proper implementation of these high technology **tools**. It is easy to lose sight of the fact that these technologies are just tools, and their utility is only as good as the scientific basis for their utilization. Although these technological capabilities are useful, they cannot supplant the need for scientific problem solving by educated and experienced criminalists.

This discussion will be fostered using "Poll Everywhere", a web-based audience response system. The audience will be able to respond in real-time to the questions posed during this presentation via the web or via SMS texting on their phones. The authors request that attendees download the Poll Everywhere free app onto their mobile devices prior to attending this presentation to facilitate discussion. This is not mandatory because attendees will be able to respond via texting.

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### **Automation, Field-Deployable Instrumentation, Technology**



### **B201 Human Factors in Forensic Science Practice Sourcebook: Beyond Bias**

*Heidi Eldridge, MS\*, RTI International, Research Triangle Park, NC 27709; John Morgan, PhD, RTI International, Center for Forensic Science, Research Triangle Park, NC 27709; Tom Busey, PhD, Bloomington, IN 47405*

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**Learning Overview:** After attending this presentation, attendees will understand: (1) the human factors that may affect forensic science practice, in addition to the well-known bias issues; (2) topics that may affect human performance, decision-making, hiring practices, and laboratory culture; and (3) a forthcoming free publication that will support the forensic science community in educating themselves about human factors in forensic science.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exposing them to areas of cognitive science that are not always considered in conversations about "human factors," which are often limited to bias topics. Managers and practitioners alike will learn that there are many human factors that may influence either individuals or systems and can affect morale, performance, and decision outcomes.

Since the 2009 National Research Council report called out a need for "research programs on human observer bias and sources of human error in forensic examinations" (p. S-18), there has been a surge in publication, discussion, and research centered on bias.<sup>1</sup> Contextual bias and confirmation bias have been the rallying cry of critics and anathema to many forensic practitioners. However, bias is not the only human factor that may influence forensic science practitioners, and while some are detrimental, others could benefit the forensic science community. For example, research demonstrates that learning is most effective when the learner is challenged with material that exceeds their current capabilities and causes errors, although much forensic training avoids tests that might reflect the failure of a student. Whether these factors are beneficial or detrimental, an understanding and awareness of human factors, and how they could affect forensic science practitioners and their work, will aid people working in forensic science.

RTI International's Forensic Technology Center of Excellence began a project in 2015 at the direction of the National Institute of Justice to assemble a Human Factors in Forensic Science Practice Sourcebook. This presentation will outline the work of the working group that was formed to achieve this task, which has met three times since then. The working group is comprised of cognitive psychology researchers and forensic scientists who have spent time getting to know one another's fields and identifying those areas where already-conducted cognitive psychology research may provide some insight into challenges faced by today's forensic science laboratories.

This presentation will also describe the structure of the resulting sourcebook, which will be freely available shortly to the forensic science community and will include chapters on: Laboratory Culture, Learning From Errors, Personnel Selection and Assessment, Communicating Forensic Science, and Accumulating, Integrating and Assessing Information.

#### **Reference(s):**

<sup>1</sup>. National Research Council. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, D.C.: The National Academies Press, 2009.

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**Cognitive Psychology, Human Factors, Bias**



### B202 Quantifying the Efficacy of Cleaning Agents for Removing Drug Background

Marcela Najarro, MFS\*, National Institute of Standards and Technology, Gaithersburg, MD 20899; Edward Sisco, PhD, National Institute of Standards and Technology, Gaithersburg, MD 20899; Amber Burns, MS, Maryland State Police Forensic Sciences Division, Pikesville, MD 21208

**Learning Overview:** After attending this presentation, attendees will understand how effective five different cleaning methods are at removing both drug particulate and drug residue from two types of surfaces.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by providing quantification values for the removal efficiency of fentanyl, carfentanil, cyclopropyl fentanyl, and other drugs from surfaces using five cleaning methods. Results from this study can assist forensic laboratories implement suitable cleaning procedures that fit their criteria.

Good laboratory practices promote cleaning procedures that help maintain workspace surfaces free of contamination after the handling of powders, packaging of evidence, etc. In the laboratory, these procedures aim to reduce exposure to analysts and maintain data integrity. In the field, decontamination efforts help render spaces safe for the public (e.g., methamphetamine remediation). Policy regarding preferred cleaning agents and cleaning frequency varies from laboratory to laboratory. The rise in cases containing highly toxic fentanyl analogs and novel psychoactive substances (NPSs) has forced forensic scientists, crime scene technicians, and first responders to consider the effectiveness of their cleaning and decontamination practices. In addition, it has been the impetus for the authors' laboratory to measure typical drug background levels within a laboratory and identify workflow processes that most contribute to the aerosolization and re-deposition of drug particulate and residue on surfaces.<sup>1</sup> Previous work in the literature shows that drug residues can persist on surfaces at police stations for some time before they sublime, decompose, or undergo photocatalysis, potentially posing an exposure risk.<sup>2</sup> The goal of this work is to validate the effectiveness of cleaning agents at removing drug background. The removal efficiency of a variety of drugs encountered was calculated to determine whether cleaning agents were better at removing a particular class of drugs.

Results from this study established quantitative values for the efficacy of five cleaning methods for the removal of particulate and residual drug samples from laboratory benches and ceramic floor tiles. Ceramic floor tiles were selected as a test surface to represent a surface first responders or hazmat teams may need to clean a spill or bulk material. The five cleaning methods examined were: methanol, soap and water, OxiClean™, adhesive and methanol, and Dahlgren Decon. Both powdered material and residues created from solution deposit were examined on the laboratory bench while only powdered material was examined on the ceramic floor tile. Quantitative analysis was completed using a LC/MS/MS. Four out of the five cleaning agents were found to have greater than 98% removal efficiency for both powders and residues. For two of the cleaning agents, whether the drug was completely degraded was also evaluated. Dahlgren Decon and OxiClean™ were evaluated not only for removal efficiency but also for the extent of chemical decomposition. Samples from ceramic floor tiles found that intact opioids could be recovered from the surfaces and the cleaning solutions, even after thirty minutes of interaction time. The results from these experiments highlight that the performance of typical cleaning agents is likely sufficient in the removal of drugs from surfaces, though additional studies in the appropriate method of implementation are required. Results from this study can assist stakeholders make data-driven decisions on optimal cleaning procedures that address their needs.

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#### Drugs, Cleaning, Quantification

## B203 False Inferences From True False and False True Positives and Negatives

Stewart Walker, PhD\*, Flinders University, Adelaide, South Australia 5001, AUSTRALIA

**Learning Overview:** After attending this presentation, attendees will be aware that sometimes a positive analysis is positive but not for the reasons suspected and vice versa. For example, drugs present in analysis but not deliberately taken, drugs absent but taken or carboxyhemoglobin present but person not alive and breathing during fire.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting instances when the analysis tests positive for the wrong reason and when the analysis tests negative for the wrong reasons.

Forensic Investigations that use scientific analysis rely on an analytical test producing a positive or a negative result where appropriate. This presentation will present examples from recent research when the analysis of evidence points one way that would lead to the wrong inference. The sampling is CORRECT. The sample preparation and analysis are CORRECT. The positive or negative results from the analysis are CORRECT the material is present or absent BUT the inference that would be taken is WRONG.

Examples are presented where:

- (1) drugs are detected in humans when they hadn't taken drugs either because of postmortem formation or due to exposure by living in a contaminated house (false positive).<sup>1-2</sup>

Post-mortem formation: Under normal circumstances there should not be any sugar or yeast present in urine. However, there are some medical conditions, including pregnancy, diabetes (hypoglycaemic incident) and Renal Glucosuria, where sugar can be in urine. If this is coupled with yeast infection and appropriate temperature postmortem formation of ethanol could result.<sup>1</sup>

Innocent Exposure: In a case study, five members of a family living in a house that had previously been used as a clan laboratory were found to have different amounts of amphetamine and methamphetamine on their hair and in their hair depending on their age, exposure, and their activities in the house.<sup>2,3</sup>

- (2) when drugs are not found when they had been taken because of postmortem microbial degradation (false negative).<sup>1,4,5</sup>

In this case a patient that was known to be prescribed specific drugs (Risperidone and Paliperidone) tested negative to both drugs after death, but investigation of bacterial degradation of these drugs and discovery of the metabolites revealed the complete degradation of these drugs in this body. Other cases had a mixture of parent drug and metabolite indicating that the amount of drug detected was not a true representation of the amount of drug taken pre-mortem.

- (3) carboxyhemoglobin levels would be interpreted to indicate a victim was alive at the time of the fire because of postmortem infusion of carbon monoxide through skin or breaches, but the victim was not alive.<sup>6,7</sup>

- (4) when the food or drink you buy isn't what it says it is on the label due to contamination with pesticides, adulterants, or due to fraudulent labelling or deliberate mis-identification of origin.<sup>8</sup>

These examples could lead to an incorrect guilty or innocent verdict. With food fraud, mothers believe they are giving sufficient nutrients, but are 'short changing' their children potentially leading to illness and even death.

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2. Wright, J.L., Kenneally, M., Edwards, J.W. and Walker G.S, Adverse Health Effects Associated with Living in a Former Methamphetamine Laboratory – Victoria, Australia, 2015, *MMWR – Morbidity and Mortality Weekly Report*, 2017, Vol 65 Issue 52 1470-1473 (Australian and New Zealand National Institute of Forensic Science Best Case Study Award 2017)
3. Wright, J.L., Edwards, J.W. and Walker G.S, Exposures associated with clandestine methamphetamine drug laboratories in Australia, *Reviews on Environmental Health*, 2016, 1 31 (3) 329-352
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5. Danielle M. Butzbach, Peter C. Stockham, Hilton J. Kobus, D. Noel Sims, Roger W. Byard, Robert J. Lokan and G. Stewart Walker, Stability of Serotonin-Selective Antidepressants in Sterile and Decomposing Liver Tissue, *Journal of Forensic Science*, 2013, 58(S1) S117-S125 (Australian and New Zealand National Institute of Forensic Science Highly Commended - Best Paper Award 2014)
6. Claire Sully, Stewart Walker and Neil E.I. Langlois, Review of autopsy reports of deaths relating to fire in South Australia 2000-2015, *Forensic Science, Medicine and Pathology*, 2018, 14(2) 180-187.



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### **False Conclusions, Fire Deaths, Drugs**





### C1 Convolutional Neural Networks for the Automatic Classification of Shoe Print Features

*Miranda R. Tilton, BA\*, CSAFE, Ames, IA 50011; Susan VanderPlas, Ames, IA 50011*

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**Learning Overview:** After attending this presentation, attendees will be familiar with the ways that Convolutional Neural Networks (CNNs) can be trained and implemented to classify forensic pattern evidence, specifically with shoe print features.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a powerful machine learning method to automatically classify shoe prints with the goal of improving the reliability of pattern evidence analysis. Results of this research can improve the speed of shoe print database searches and provide valuable information about the relative frequency of specific characteristics found on shoe prints.

The improvement of modern computing has led to the widespread implementation of machine learning, in which computers “learn” about data without being explicitly programmed. CNNs are a form of deep learning that mimics the human brain by using complex networks of hidden layers to learn patterns and make decisions about the contents of an image. For example, we may see an image that contains features such as “eyes” and “nose” and conclude that the image contains a “face.” Similarly, CNNs learn and combine local patterns in a set of training images to learn to detect the presence of a desired set of features in new images.

In forensic applications, images of shoe prints are well-suited to classification using CNNs. Shoe prints often contain distinct and well-defined shapes, such as circles and triangles, as well as text and textures that can be easily classified by a CNN. Once the shapes contained in a print are detected and classified, the resulting labels can be combined to determine the shoe’s category (e.g., athletic, formal) and brand. This automatic classification can be used to speed up database searches for a given shoe print and can also be used to determine the relative frequency with which certain characteristics appear in shoes that are commonly worn within specific populations. Development of a model that can automatically identify class characteristics of shoes and prints will provide a valuable tool for examiners to use when searching for candidate shoe models which may match a questioned print. In addition, various statistical methods for assessing class characteristic frequency within a population depend on being able to automatically identify class characteristics, allowing for large amounts of data collection. CNNs for class characteristics represent an important first step toward greater statistical quantification of class characteristic frequency within a local population.

Using approximately 2,000 images of shoe soles of various types, brands, and sizes, the presented model can successfully identify several different class characteristics with accuracy above 95%.

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#### Machine Learning, Shoe Print, Pattern Evidence



C2 WITHDRAWN



### C3 Improving Information Sharing With the Cyber-Investigation Analysis Standard Expression/Unified Cyber Ontology (CASE/UCO)

Vikram Harichandran, MS\*, Fairfax, VA 22031; Cory Hall, MS, The MITRE Corporation, Severn, MD 21144

**Learning Overview:** After attending this presentation, attendees will have a deeper understanding of what an ontology is, why adoption of the CASE standard is different from other specifications, why it will massively improve information sharing, and where the proper resources are for their organization to get involved in development or adoption.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how CASE can be adopted and integrated to better share information, also allowing those involved to help shape the ontology to cover gaps.

As the cybersecurity domain has grown, the amount of increasingly varied information needing to be shared has increased. There is now a greater need to validate, normalize, combine, and correlate investigative data between different countries, domains, organizations, teams, individuals, classification levels, and tools; the status quo is insufficient.

CASE is an international open-source and community-developed ontology/specification language that seeks to cover this gap in the most inclusive manner possible.<sup>1</sup> Work on what eventually became CASE began in 2015, and the project now involves more than two dozen public organizations.<sup>2,3</sup> It derives from UCO and is thus formally cited as CASE/UCO. UCO is intended to allow compatibility between CASE and other preexisting ontologies/schemas. However, unlike prior domain-specific specifications like Structured Threat Information Expression (STIX) and Digital Forensics Analysis eXpression (DFAX), CASE attempts to bring domains together, including incident response, counterterrorism, criminal justice, forensics, intelligence, and situational awareness. This will enable better workflow efficiencies in laboratories, cross-correlation between investigations under different jurisdictions, potentially on the same malicious actors, and a more aware view of criminal patterns.

The CASE team facilitates integration of subdomain knowledge from its global academic, private sector, and government community members; the ontology retains a core focus on tracking provenance and casework metadata (e.g., people who performed an action using a specific tool). Linked-data in the form of Resource Description Framework (RDF) graphs are used to export all data as JSON-LD (JSON for Linked-Data), which can be stored for transit, archiving, or tool ingestion. This past year, the MITRE Corporation has assisted in improving documentation and the supporting framework, while both European Union and United States governments have begun discussing a mandate for widespread adoption. The Github repositories (<https://github.com/ucoproject>) provide proof-of-concept mappings and implementations into forensics tools and outline the details of using the Application Programming Interfaces (API) in different ways. Additionally, exploration tools are available for the Terse RDF Triple Language (Turtle) format that the ontology is specified in. As the ontology evolves, it will encompass perspectives from community popular votes. However, custom and private schemas may still be supported for cases in which private or government tools desire integration with a preexisting data model.

This presentation will include a technical overview and example implementations of CASE, including a glance at the Python API and other resources.

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#### Information Sharing, Ontology, Standards



### C4 Inter-Regional Digital Forensic Knowledge and Information Exchange Platform

Eoghan Casey, PhD\*, University of Lausanne, Lausanne, Vaud, SWITZERLAND; Anna Zehnder, Unil, Lausanne 1005, SWITZERLAND

**Learning Overview:** After attending this presentation, attendees will understand: (1) the distinct challenges and requirements associated with sharing information and knowledge across digital forensic practices; (2) the scope of digital forensic information and knowledge to be shared; (3) the sharing priorities as expressed by practitioners; and (4) the challenges of establishing a maintainable inter-regional knowledge management system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing a framework for building an inter-regional digital forensic knowledge and information exchange platform that helps fill knowledge gaps by providing digital forensic scientists easy access to needed information. Such a platform also helps stop knowledge drain by capturing solutions in a form that others can reuse in future cases.

Digital forensic capabilities are being put in the hands of individual investigators, enabling them to process evidence with little assistance from digital forensic science laboratories. As a result, each situation or problem/solution is isolated to the context of an individual investigation. This isolation reduces sharing of knowledge between entities (knowledge gap), reduces curation of knowledge and expertise (knowledge spillage), and reduces visibility across cases (repetition blindness). Forensic science laboratories are in a strong position to address these challenges by playing a pivotal role in managing and disseminating knowledge among digital investigators they support and maintaining the quality of forensic results in a decentralized environment.<sup>1</sup>

It is infeasible for a single digital forensic scientist to know about all advances in technology, new methods, and uses of digital evidence. When they encounter a new situation, they might not know about relevant processes or tools that have already been developed and are fit-for-purpose in that specific situation. This knowledge problem can result in digital forensic scientists missing relevant information or misinterpreting important evidence. Mistakes and missed opportunities in digital investigations can have severe consequences, including imprisoning innocent people, dangerous criminals remaining free to commit additional offenses, or continued victimization of the organizations and people targeted by offenses.<sup>2</sup>

Digital forensic science laboratories have the opportunity (perhaps even a duty) to mitigate these problems by systematically distilling and circulating knowledge throughout the decentralized forensic ecosystem.<sup>3</sup>

This work details the design and related challenges of an inter-regional digital forensic knowledge management platform called the Knowledge and Information Exchange Platform (KIEP). This work takes a bottom-up approach, incorporating input from dozens of digital forensic practitioners in an effort to identify common needs and general priorities.<sup>4</sup> The results encompass a wide range of issues related to codifying and sharing digital forensic knowledge, including skills, processes and tools. KIEP is motivated in large part by the need in digital forensic science to improve collaboration and communication between investigators and digital forensic scientists, and to keep pace with new technologies and large quantity of data.

The sustainability of such a consolidated knowledge management platform is also discussed, with mechanisms to motivate digital forensic scientists to share their knowledge.

The impact of this work includes: (1) saving time and money and increasing the efficiency of processing digital traces; (2) reducing missed opportunities to utilize digital traces + the risk of overlooked evidence; (3) reducing wasted resources (duplication of effort, reinventing the wheel); (4) reducing the frustration of processing digital traces + increasing collaboration; (5) increasing consistency and repeatability of digital forensic results; and (6) strengthening digital forensics with knowledge from forensic science and intelligence.

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1. Casey E., Ribaux O., Roux C. The Kodak Syndrome: Risks and Opportunities Created by Decentralization of Forensic Capabilities. *J Forensic Sci.* 2019 (In press - <https://doi.org/10.1111/1556-4029.13849>).
2. Casey E. *Reinforcing the Scientific Method in Digital Investigations Using a Case-Based Reasoning (CBR) System*. PhD dissertation. Belfield, Dublin: University College Dublin, 2013.
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4. Zehnder A. *Systematic Management and Exchange of Digital Forensics and its Investigation Knowledge Between Swiss Police Agencies*. Master's thesis. Batochime, Lausanne: University of Lausanne, 2018.

#### Digital Forensic Science, Knowledge Management, Information Sharing



### C5 Digital Trace Reference Library (DTRL)

*Eoghan Casey, PhD\*, University of Lausanne, Lausanne, Vaud, SWITZERLAND; Owen Brady, PhD, KCL, London WC2R 2LS, UNITED KINGDOM*

**Learning Overview:** After attending this presentation, attendees will understand how to structure digital traces in a database in a manner that can be readily referenced by practitioners or queried automatically by tools. Attendees will also learn how digital traces are represented using the evolving Cyber-Investigation Analysis Standard Expression (CASE) standard.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how the DTRL can advance digital forensic tool validation and R&D in digital forensics.

The DTRL implements an ontology-based classification system to help users find digital traces that are relevant to their inquiry and to support systematic analysis of digital traces. The structure of the DTRL enables automated queries to augment tool testing and evidence processing.

The importance of reference databases in forensic science was raised in the President's Council of Advisors on Science and Technology (PCAST) report on forensic science. In some respects, the DTRL is comparable to the Ballistics Toolmark Database with standardized representation of characteristics using an open-source data format.

The DTRL is built on the Digital Evidence Semantic Ontology (DESO) and the Cyber-investigation Analysis Standard Expression (CASE). This open-source ontology-based model is designed to standardize and extend emerging proprietary artifact recording systems, including the University of New Haven's (UNH) Artifact Genome Project (AGP), Magnet Forensics' Artifact Exchange, and Google's® Forensic Artifacts.

The DTRL aims to address the following issues: (1) availability—what artifacts are available on a particular digital evidence source?; (2) selection—from the perspective of the investigation, which ones are required?; (3) correlation—allowing the effective comparison of artifacts from disparate sources to make evidential connections; and (4) reliability—what is the basis behind a particular artifact?

Using CASE to represent digital traces in the DTRL allows any tool to parse the standard structures for a variety of purposes. Digital forensic tools can be tested to determine which indexed entries in the DTRL are fully/partially supported by each tool. For each digital trace, the DTRL cites supporting documentation and/or research results, which provide additional information for developers and practitioners to learn more about specific digital traces. This information is useful for tool development purposes and explaining digital evidence in court.

Specifically, the ontology has three classes: (1) location—the actual pieces of data. These could be pertinent to a boot record, file system, operating system or application file, such as a SQLite Database. In much the same way that Advanced Forensic Framework 4 (AFF4) breaks down an image into its component parts and prioritizes, so does DESO. In fact, it's interesting how the two could be paired together; (2) Type Identifier—each of the trace locations is assigned a Type Identifier that allows identification of common artifacts, irrespective of source. This allows the discovery of connections that had not previously been considered. For example, USB serial number, so that those with a common type can be compared. This also allows for a common reporting format to aid comparison; and (3) provenance—the basis for stating that the data at the stated location is the particular Type Identifier

The DTRL overcomes limitations in emerging proprietary systems in the following ways: (1) expressivity—the ontology-based structure of the DTRL clearly differentiates between multiple facets of a digital trace, whereas other systems do not differentiate clearly between the different facets. In UNH's AGP, an entry that is classified as a File object can also contain a username and password. UNH's AGP uses tags on the File object to label such important characteristics rather than distinctly representing these characteristics and their relationship with the File object. Simply put, in general, the AGP does not capture artifacts, but the File in which they are contained. The AGP user is then required to discover what piece of data within the File is relevant and what the data represents. This lacks precision and does not provide the required coordinates for automation; (2) non-ambiguity—the ontology-based structure of the DTRL represents digital traces and their context in a non-ambiguous manner, whereas other systems have ambiguity. For instance, searching UNH's AGP for Media Access Control (MAC) addresses using "MAC" on its keyword and tags function also returned hits relating to "Macintosh." Some users might misinterpret the "MAC" tag as Message Authentication Code; and (3) extensibility—CASE can support many kinds of digital trace, and can be extended to represent new traces as needed.

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#### Digital Traces, Digital Forensic Standards, Tool Testing and Validation



### C6 The Development of Protocols for the Analysis of Mobile Devices That Have Been Submerged in Oil-Based, Flammable, and Clandestine Chemical Liquids

*Nico Kresl\*, Huntington, WV 25704; Chelsey McPhillen\*, Port Washington, OH 43837; HollyAnn D. Swann, BA, MUFIA, Huntington, WV 25701; Steven B. Watson, BA, VTO, Broomfield, CO 80021-8042; David Rathbone, VTO, Broomfield, CO 80021; Lauren L. Richards-Waugh, PhD, Marshall University Forensic Science Program, Huntington, WV 25701; Joshua L. Brunty, MS, Marshall University, Huntington, WV 25701; Catherine G. Rushton, EdD, Marshall University Forensic Science Program, Huntington, WV 25701*

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**Learning Overview:** After attending this presentation, attendees will understand the protocols that were developed to aid in the handling of mobile devices that have been submerged in either oil-based, flammable, or clandestine chemical liquids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing techniques to safely handle and acquire data from mobile devices that have been damaged with either oil-based, flammable, or clandestine chemical liquids.

Society has become inundated with a large variety of connected mobile devices used in the course of everyday life, and these devices become useful during forensic investigations. Mobile devices found in their original state or submerged in water-based liquids are examined by the guidelines established by the individual labs or their accrediting body. However, mobile devices that have been submerged in non-water-based liquids tend to be neglected in some laboratories due to not having the proper protocols and procedures in place to inform their examiners on safe and proper handling. These damage modalities, which tend to result in the mobile device being turned away, include, but are not limited to, oil-based liquids, flammable liquids, and clandestine chemical liquids. The overall goal of this project was to develop a protocol that can be used by the average digital forensic laboratory so potential items of high evidentiary value are not turned away due to a lack of knowledge in how to safely handle the device.

In conjunction with VTO Labs, Marshall University's Forensic Science Center endeavored to create protocols to demonstrate how mobile devices damaged with either oil-based, flammable, or clandestine chemical liquids should be handled to ensure the safety of the examiner and the data on the mobile device. The mobile devices were split into three equal groupings of devices and every device that could be imaged was imaged. Imaging was carried out via cable acquisition with Cellebrite®, UFED 4PC®, and SHA256 hash values were generated for each image. After imaging, the phones were submerged in their assigned liquid for a total of seven days before they were removed and neutralized, if necessary, before they were disassembled for the ultrasonic cleaning process. Ultrasonic cleaning was conducted using ElmaTec®, Clean A1®, and Clean S1® detergents with a modified manufacturing industry standard (IPC/JEDEC Standard 610) that VTO Labs had used in previous studies to great effect. Confirmation that the devices were cleaned of all remaining residue was conducted via Gas Chromatography/Mass Spectrometry (GC/MS) for the oil-based liquids and the flammable liquids using ASTM E2881-13e1 and ASTM E1618-14 methods, respectively. Confirmation that the devices submerged in clandestine chemical liquids was performed by using litmus paper to indicate a neutral pH value. If any cleaning methodology did not result in satisfactory results, additional cycles or pre-work steps were utilized to achieve the best result. To ensure the data remained intact after cleaning, the devices were reimaged using cable acquisition with a Cellebrite®, UFED 4PC®, or via chip-off methods. The SHA256 hash values generated after submersion and cleaning were compared with the SHA256 hash values generated prior to submersion.

*The funding for this research was sponsored by the United States Department of Homeland Security (DHS) Science and Technology Directorate, Cyber Security Division (DHS S&T/CSD) via contract number HHSP233201700017C, which was awarded to VTO Labs.*

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#### Mobile Forensics, Damaged Devices, Device Cleaning



### C7 Guidance to Digital Forensics Practitioners on the Handling of Evidence Exposed to Biohazardous Materials

*Steven B. Watson, BA\*, VTO, Broomfield, CO 80021-8042; Kaitlyn Fox, BS\*, VTO, Inc, Broomfield, CO 80021*

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**Learning Overview:** The goal of this presentation is to provide guidance to digital forensic practitioners and digital forensic labs on the best practices for handling evidence exposed to biohazardous materials. Limited guidance exists today as to how digital forensic practitioners should protect themselves when encountering devices exposed to biohazardous materials.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing guidance to digital forensic practitioners on the best methods for protecting themselves when handling evidence exposed to biohazardous materials. This presentation will provide guidance to practitioners, management, and labs on the correct personal protective equipment, hazardous cleanup materials, disposal, and evidence packaging to have, should it be needed.

**Objectives:** (1) identification of standards relevant to biohazardous material handling; (2) understanding of universal precautions; (3) identification of types of hazards; (4) understanding of bloodborne pathogens; (5) understanding of other biohazardous materials; (6) Personal Protective Equipment (PPE) levels; (7) decontamination of items exposed to biohazardous materials; (8) implication of decontamination steps on items of electronic evidence; (9) explanation of Safety Data Sheets (SDS); and (10) best practices for handling electronic evidence items exposed to biohazardous materials.

In the course of establishing procedures and best practices for addressing damaged devices, the research studies undertaken encountered a gap in lab safety policies. The gap was how to address physical items of evidence that may have been exposed to biohazardous materials. A survey of digital forensics labs in local, state, and federal law enforcement agencies identified that most agencies did not have personnel safety protocols to be used for handling evidence once it arrived in the lab.

While crime scene investigators don PPE when collecting evidence on scene, the same items of evidence may be delivered to a lab with no biohazard labeling nor contained in a biohazard-safe container. The safety disconnect extends beyond labeling and biohazard packaging for delivery to the lab.

Lab personnel may lack clear direction regarding safely handling devices during evidence processing, cleaning biohazardous materials off devices, and which PPE to wear to protect themselves while the device exists in the lab.

Industry standards in other scientific disciplines exist with direct applicability to the discipline of digital forensic science. This presentation will explore, identify, and highlight those techniques through literature review and propose the relevant techniques for addressing digital evidence with safety for the handler and care for the discreet pieces of digital evidence.

This presentation will also review the industry standard product SDS (formerly known as material safety data sheets) to highlight how the sections of the sheet can guide practitioners on the appropriate PPE, handling, storage, and spill methods to use in the event these materials are introduced into their labs.

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#### Digital Forensics, Biohazard, Bloodborne Pathogens



### C8 Examination of First-Year Research Findings for the Safer Viewing Platform: A Convergence of Convolutional Neural Network Predictions and a Safer Viewing Technology to Speed Up the Triage of Sex Assault Multimedia While Managing the Investigator's Stress Level

*Cory Hall, MS\*, The MITRE Corporation, Severn, MD 21144; Jay Doyle, PhD\*, The MITRE Corporation, McLean, VA 22102; John Tavolacci, BS\*, The MITRE Corporation, McLean, MD 22102; James D. Morris-King, PhD, The MITRE Corporation, McLean, MD 22102; Rob H. Schmicker, BS, The MITRE Corporation, McLean, MD 22102*

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**Learning Overview:** After attending this presentation, attendees will understand the stressors and technological demands placed on digital investigators who conduct sexual assault cases. Knowledge gained through interviews with digital investigators and their managers from several local, state, federal, and international jurisdictions regarding conducting sex assault digital investigations will be presented. Attendees will learn about the observed effectiveness of applying deep learning technology to triage large datasets of sexually explicit materials, as well as the techniques to reduce the exposure of this content to digital investigators during the triage examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the ability and effectiveness in conducting multimedia analysis by using deep learning techniques to give structure to previously unstructured multimedia content. This technique may identify investigative leads more quickly than using traditional file system forensic techniques.

A multidisciplinary team from the MITRE Corporation built a proof-of-concept technology in 2017 and 2018, named the Safer Viewing Platform. The Safer Viewing Platform is a technology that combines an ensemble of convolutional neural networks, a Safer Viewing Layer, and a rapid imaging and carving technology. This study used a simplified workflow that focused on analyzing the multimedia directly, rather than conducting a lengthy traditional file system examination. The team evaluated a tasking and retrieval mechanism that lets the digital investigator ask the Safer Viewing Platform to find specific content based on a custom query of age and gender estimation, display of nudity, face identification, and image or video frame scene attributes. This study experimented with random datasets that represent sex assault investigation data with as many as 500K multimedia files. This study worked with federal law enforcement to evaluate key parts of the Safer Viewing Platform on real sex assault content. It was found that it is possible to produce sex assault automated investigative leads based on the digital investigators initial query. It was observed that an Android™ (16GB) phone with 43K images could be processed using this technique, and that previously unknown sexual assault content can be found within 20 minutes from the start of acquisition. Further, this study developed a new concept called the Safer Viewing Layer, which creates a localized transparent layer over the nudity region in a digital image or video frame. This presentation will report on findings from an on-going pilot of the Safer Viewing Layer with federal digital forensics examiners. The Safer Viewing Layer experiment is designed to evaluate the effectiveness of the technology to help the examiner manage their stress level during the triage process of sex assault content.

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**Safer Viewing, Multimedia Analysis, Sexual Assault**





### C9 Human Versus Computer: Age Estimation Applied to Child Pornography

Mateus D.C. Polastro, MSc\*, Brazilian Federal Police, Campo Grande, Mato Grosso do Sul 79110-503, BRAZIL

**Learning Overview:** After attending this presentation, attendees will understand some of the challenges related to the identification of child pornography images and will learn some promising technologies that can be used against the advancement of this type of crime.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comparative study of the ability to estimate people's ages using computer vision and pattern recognition versus the human ability to predict the age. In addition, this presentation will open a discussion concerning automatic age estimation for cases of child pornography and its impact.

Worldwide law enforcement is struggling to combat child pornography. With the increasing use of computers, cell phones, the internet, Deep Web, and social networks, among others, it is very common to find photos and videos stored in digital format on computers and cell phones. Thus, in investigations of child pornography, it is necessary to analyze thousands or even millions of images.

When an investigator is looking for child pornography images, the first step is to find images containing nudity or sex scenes. A person can perform this step visually or even in an automated manner.<sup>1,2,3</sup> After that, the task is to identify the participation of at least one child in the image. The presence of adolescents is also important in child pornography cases, since there are punishments in most countries. The possibility of automating the age estimation step using available age estimation algorithms may be an alternative in helping to decrease digital forensic backlogs related to child pornography. In addition, it can minimize the negative impact of exposure to investigators from child pornography images.

To identify the human ability to estimate age from photos, a survey was conducted in which respondents would estimate the age of 47 people displayed in pictures.<sup>4</sup> The photos were of people of both genders who were between 0 and 25 years of age. The images were in uncontrolled scenes, contained no nudity, and the respondents would estimate the age based only on face and body proportions.

With the goal of comparing the automatic age estimation with the results obtained in the study described above, the same 47 images were submitted to some state-of-the-art age estimation services: Microsoft's<sup>®</sup> how-old.net, Face ++<sup>™</sup>, and VeriLook SDK<sup>™</sup>.<sup>5-7</sup> The result obtained by each algorithm was then compared to the answers provided by the survey respondents. It is important to note that in cases related to child pornography, generally, the main goal is to locate the presence of a child or adolescent in the image, and it is not necessary to define the exact age. Thus, three comparison groups were created: (1) people under 14 years of age; (2) people between 14 and 18 years of age; and (3) people over 18 years of age. The results of the estimates made by the people and those made by the algorithms were considered an error when the person's real age was in one of these groups but was not estimated in the correct group. Additionally, in some cases, the algorithms did not detect faces in any images, so it was not possible to estimate the age, and these were not counted as errors.

The findings of this research demonstrate that age estimates made by the survey respondents were more accurate than those performed by the tested algorithms. On the other hand, the algorithms did not classify any person of group 3, in group 1, or in group 2; that is, no false positive, unlike the estimates made by the survey respondents, who misjudged 33% of the cases. The highest error rates occurred in group 2, both by algorithms and by the survey respondents. It was expected the error rate would be higher in this group since adolescents may often have characteristics that confuse them with adults.

The results of this study suggest that algorithms have not yet outperformed humans in estimating people's age. Nevertheless, there has been much progress in the accuracy of the classifiers, and it is already possible to use them in real cases in which false negatives are accepted. Detailed results will be shared at this presentation and recommendations for further research will be discussed.

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5. Microsoft<sup>®</sup>. <<https://how-old.net>>.
6. Face++<sup>™</sup> api. <<http://www.faceplusplus.com>>.
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#### Age Estimation, Child Pornography, Nudity Detection



### C10 Assessing the Psychological Well-Being of Multimedia Forensic Analysts

Kathryn C. Seigfried-Spellar, PhD\*, Purdue University, West Lafayette, IN 47907; Kyle A. Schnetzler, BS, Purdue University, West Lafayette, IN 47907; Marla E. Carroll, BS\*, Forensic Video & Audio Associates, Inc, Plantation, FL 33317

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the psychological well-being of multimedia forensic analysts exposed to evidence from different types of criminal cases, as well as the availability of mental health services.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining the psychological impact on multimedia forensic analysts who work different types of criminal cases.

Nearly every criminal investigation involves more than one form of digital evidence. In some cases, multimedia forensic examiners need to enhance audio and video evidence in order to identify the actors or clarify the context of the situation. Examiners may also need to compare/analyze clothing, tattoos, or individuals, which may involve viewing or hearing heinous acts (e.g., homicide, child sex abuse, torture). Research indicates criminal justice occupations are associated with high work-related stress leading to psychological illness and high burnout rates, such as investigators or digital forensic examiners working child pornography investigations and 911 dispatchers who respond to emergency phone calls.<sup>1-3</sup> However, there has not been any research that has examined the psychological well-being, job-related satisfaction, stress related to working specific types of cases, and availability of mental health services for multimedia (audio/video/image) forensic analysts. For example, do analysts experience more stress as a result of working specific cases or crimes (e.g., child sex abuse vs. homicide), and are there differences on psychological well-being for audio analysts vs. video analysts? The overall goal is to better understand the well-being of multimedia forensic analysts exposed to evidence from different types of criminal cases.

This current study was the first to compare the psychological well-being and work-related stress experienced by multimedia forensic analysts. The anonymous internet-based survey was hosted on Qualtrics®; respondents were solicited through professional organizations for multimedia analysts, and members were encouraged to share the link with fellow analysts (i.e., snowball sampling). One hundred thirty-six respondents completed the anonymous internet-based survey assessing psychological well-being, job satisfaction, stress related to the type of criminal cases worked (e.g., child sex abuse, elderly abuse), and availability of mental health services. Respondents were compensated with a \$10.00 Amazon® e-gift card by completing a separate, independent survey that collected only an email address—it was not possible to link the responses from the two surveys.

Based on their current self-reported duties, 67 respondents were identified as audio analysts; 126 as video analysts; and 68 as image analysts. In addition, 27 respondents reported that they also worked as an investigator or detective. Each duty was coded dichotomously (Non-Audio vs. Audio analysts). It was possible for respondents to self-report multiple duties (e.g., working as both an audio and video analyst). The results suggested significant differences between multimedia forensic analysts on psychological well-being and job satisfaction. In addition, the results varied when controlled for the role of detective. The results will be fully discussed as well as suggestions for future research and policy implications regarding mental health services for multimedia forensic analysts.

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**Mental Health, Multimedia Forensics, Digital Forensics**

### C11 A Fake News Reporting Manual to the Elections

*Mateus D.C. Polastro, MSc\*, Brazilian Federal Police, Campo Grande, Mato Grosso do Sul 79110-503, BRAZIL; Pedro M.S. Eleuterio, MSc, Brazilian Federal Police, Campo Grande 79040010, BRAZIL*

**Learning Overview:** After attending this presentation, attendees will understand some of the challenges related to the identification of posts on social networks and news distributed through messaging apps and their potential impact on elections.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a manual to help citizens report fake news propagated through social networks and messaging apps, allowing law enforcement to respond more effectively to complaints.

The advancement and democratization of the internet have made it easier for people to access various types of information. However, information obtained in this way can be false or distorted, and users can be presented with many difficulties in differentiating it from the truth.

With the increasing use of social networks, sharing all types of information has become very common, including the well-known “fake news.” The dissemination of fake news through social networks such as Facebook™ and Instagram™, for example, happens very quickly because the trust between people in these networks creates an idea of credibility that, in addition to influencing the recipient, also provides a greater chance for sharing. Furthermore, people use social networks as a source of news and, beyond that, messaging apps have been gaining ever more space as a source of news and information, which aggravates the problem.<sup>1-3</sup>

Because of this power to transmit news to people, social networks and messaging apps have been heavily used to influence politics based on fake news, directly affecting democratic societies. There are several reports of cases in which fake news had influence on European and American elections.<sup>4</sup>

The strategies used to create fake news are diverse. These strategies range from contracting marketing agencies to create news and memes to denigrate a candidate’s image to technological ones, such as using robots to quickly propagate fake news and provide credibility to dubious posts on social networks. An attempt to curb the spread of such fake news can be made through denunciations by citizens or committees of political parties. Such complaints must identify the following posting artifacts to allow tracking: (1) content; (2) date and time; and (3) user name or other identification. Although these artifacts represent simple information, sometimes they do not contain all essential data for law enforcement, harming the identification of the post, its author, and even withdrawal from circulation, making tracking impossible.

To enable fake news reports to be forwarded to law enforcement with all the information necessary for the investigation of electoral crimes, a manual has been created to assist whistleblowers in reporting fake news propagated through the following social networks and messaging apps: Facebook™, Instagram™, YouTube™, Twitter™, WhatsApp™, and Telegram™. This manual was freely available to the Brazilian population on the websites of the institutions responsible for the elections.

Through this manual, it is possible for the complainant, even if unfamiliar with social networks or messaging apps, to collect the necessary information for proper tracking, such as post content, profile identification or user name, and dates, among other identifying data. The entire manual uses simple language and detailed illustrations of the steps required to obtain the relevant data in each of the most-used social networks or messaging apps in Brazil. Moreover, it suggests a better way of presenting the information obtained to law enforcement, whether in electronic or printed form.

With the use of the manual, the handling of complaints became faster and more effective, as law enforcement started to track posts and authors more quickly.

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3. Nic Newman. *Overview and Key Findings of the 2018 Report*. Accessed July 25, 2018, <http://www.digitalnewsreport.org/survey/2018/overview-key-findings-2018>.
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#### Fake News, Social Networks, Messaging Apps

### C12 Social Network Image Ballistics Through Automatic Reverse Engineering

*Oliver Giudice, PhD\**, Roma 00044, ITALY; *Antonino Paratore, MS\**, ICT Lab S.R.l. Spinoff of Università di Catania, Catania 95125, ITALY; *Sebastiano Battiato, PhD\**, Università di Catania, Catania 95125, ITALY; *Luca Guarnera, MS\**, Università di Catania, Catania 95125, ITALY

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**Learning Overview:** After attending this presentation, attendees will be aware of new possibilities in digital images forensic analysis—the so-called social image ballistics, a technique that derives its name from the traditional ballistics science that attempts to identify the weapon that exploded a certain projectile, exploiting traces left on the projectile itself or on cartridges. Attendees will discover how to reconstruct the history of a digital image by understanding how they are manipulated by different social networks. Moreover, attendees will be able to identify the device that uploaded the original image and the corresponding timeframe.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a key aspect for different fields of investigation (e.g., child pornography, terrorism, etc.) and, in general, for all investigations in which it is necessary to verify the origin of an image to the user profile who published it.

The software utilized by social networks is known to alter images for bandwidth, storage, and layout reasons. Recent works have demonstrated that, although the platform heavily modifies an image, this processing leaves traces on the image itself.<sup>1-4</sup> Previous works have discussed those alterations and proposed a classification solution able to identify whether an image was processed by one of the ten social network services taken into account.<sup>4</sup>

Understanding the origin of a digital image in the era of social networks means analyzing all traces left by the social network software. Every component of a digital image will be discussed and the corresponding manipulation performed by ten social networks will be presented (filenames, Jpeg-structures, meta-data). This process may be considered a reverse engineering of social network image processing modules. Toward this goal, an automatic reverse engineering solution will be discussed.

The Classification Engine solution limit lies in its reference dataset, where the images analyzed presented fingerprints of social networks related to the time at which the dataset itself was built.<sup>4</sup> Social networks are always changing their software functioning and parameters. Thus, the dataset on which the classification engine was built could become obsolete (and in turn the Classification Engine as well). To mitigate this, it is possible to build a software solution (Continuous-Collecting Social Images) able to automatically and periodically conduct the collection and reverse engineering analysis of a social network by storing the images and all collected meta-data in a specific database and extracting new fingerprints. The automatic image collection performed during a period of one year allowed improvement in the results achieved by the Classification Engine, not only for the classification task, but also for developing additional information regarding the timeframe in which an image was supposedly uploaded.<sup>4</sup>

Finally, a use-case scenario of this type of digital image analysis will be presented and applied to the forensic and investigative domains.

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#### Social Media Ballistics, Multimedia Forensics, Source Identification



### C13 Child Pornography in Computer Forensics: What Are the Most Relevant Pieces of Digital Evidence?

*Pedro M.S. Eleuterio, MSc\*, Brazilian Federal Police, Campo Grande 79040010, BRAZIL*

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**Learning Overview:** After attending this presentation, attendees will understand the essential evidence needed in a computer forensic analysis involving child pornography, including shreds of digital evidence of illegal file possession, sharing, and production. Furthermore, in these cases, the intention of the user to reach these files is one of the most important items for the computer forensics expert to prove.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reviewing the most important pieces of digital evidence in cases of child pornography, which computer forensics experts must look for to make the forensic analysis. This presentation includes computer forensics knowledge regarding peer-to-peer tools, image and video metadata, and computer forensic analysis.

Computer forensics involving child pornography is increasing year by year. This type of digital evidence is crucial for computer forensics experts to be able to unravel related crimes, considering their country's laws. The possession of child pornographic files and their sharing are crimes in many countries. Therefore, in a computer forensic analysis, there are many pieces of digital evidence that experts must find. This study reveals some of the most relevant digital evidence in cases of child pornography in computer forensics.

First, the forensic expert must look for images and videos of child pornography stored in the digital devices. Thus, it is possible to use techniques such as comparing hash values, searching for common pedophilia keywords, using nudity detection in images, and even motion detection in videos. The EXchangeable Image File (EXIF) metadata information in images can be useful in determining if the user device has produced some pictures.

After locating the possession evidence, the computer forensics expert must look for file sharing evidence, analyzing logs of: (1) peer-to-peer programs (such as Kazaa™, Shareaza™, Ares™, Limewire, uTorrent®, eMule™, BitComet®); (2) instant messaging programs (such as WhatsApp Web, Skype®, ICQ, Google Talk®, Facebook® Messenger™); (3) web browsers' histories (such as Chrome®, Internet Explorer®, Firefox®); (4) e-mail programs and webmail caches (such as Google® Mail™, Outlook®, Thunderbird™, Hotmail®, Yahoo!®); and (5) programs to access the Dark Net and Deep Web (such as Tor™ Browser, Onion Browser™). In fact, there are many programs to download and upload files, which can be used to share illegal content. Therefore, the study of the newly available tools is always crucial to the forensic expert.

However, one of the most relevant forms of evidence in these cases is to prove the intention of the user to obtain this kind of illegal content. Therefore, the forensic expert must search for the keywords used to reach these illegal files, like Web browser keyword/form list, keywords of sharing programs and visited sites, among others. In some cases, the forensic expert may find texting, sexting, and child grooming evidence, which are imperative in cases of child exploitation and child sexual abuse.

The evidence found in digital devices related to child pornography is very relevant to the solution of these cases. There are many ways to find child pornography evidence in digital devices and the computer forensics experts must be aware. After all, in some cases, the computer forensic analysis can be the only method to discover the sexual abuse of children, allowing the aggressors punishment.

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#### Computer Forensics, Child Pornography, Pedophilia



### C14 Fortnite Forensics: A Study of How to Extract Artifacts From Android™ Memory

*Justin Grover, MS\*, The MITRE Corporation, Mclean, VA 22102; Chris Meffert, MS\*, The MITRE Corporation, McLean, VA 22102*

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**Learning Overview:** After attending this presentation, attendees will better understand the current state of performing memory forensics on Android™ devices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing techniques for researchers to more effectively perform forensic explorations of mobile applications.

The popular Fortnite application will be used as an example to explain the procedures involved with dealing with Android™ memory and the current challenges facing the community. Fortnite, due to its design and scope as a gaming application, may not result in many artifacts of forensic interest; however, the focus of this presentation is not on results but on the involved process used to derive any results. The forensic community will benefit by realizing the state of the field, how Android™ memory forensics can be applied to investigations, and how this process can be used to leverage information from other, more forensically valuable, applications of interest.

Memory forensics in recent years has become more commonplace in forensic and incident response investigations involving traditional laptops and desktops running Windows®, Mac OSX®, or Linux®. Procedures and tools are widely available on these platforms, and many investigators realize that “pulling the plug” will result in potential data losses. However, memory forensics on mobile devices is not a regular practice in current investigations. Given the widespread nature and usage of mobile devices across the world and their enormous impact on investigations, this presentation seeks to explore why memory forensics is not a common aspect of mobile device forensics.

The state of the mobile memory forensics field will be conveyed to attendees by summarizing recent research efforts, explaining hands-on experimentations with various memory extraction methods, and providing an assessment on current memory examination and analysis options within the popular analytical Volatility and Rekall frameworks.<sup>1,2</sup>

This presentation will also introduce attendees to Frida, a dynamic process injection (also known as “hooking”) toolkit that can assist researchers and reverse engineers with performing memory analysis on mobile operating systems and their running applications.<sup>3</sup> This tool can be used to study artifacts that are stored in application memory or on a device’s flash storage.

Finally, some brief thoughts will be offered on the state of memory forensics on the iOS® platform and why it is such a challenge for security researchers and investigators.

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**Memory Forensics, Android™, Forensics**



### C15 WAEventsLogParser: Recovering Hidden (and Relevant) Evidence From WhatsApp Events Log File in Android™ Devices

Mateus D.C. Polastro, MSc\*, Brazilian Federal Police, Campo Grande, Mato Grosso do Sul 79110-503, BRAZIL

**Learning Overview:** After attending this presentation, attendees will be aware of the most relevant evidence stored in the WhatsApp events log file and will understand how investigators can use them for forensic purposes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting and exploring a new source of evidence of activities on WhatsApp for Android™, the event log file. It will also present the software developed to interpret these events and will show how new events can be discovered and configured.

Mobile messaging apps are very popular around the world and WhatsApp is the most used when considering the number of active users.<sup>1</sup> WhatsApp ended the year 2017 with 1.5 billion users, and many countries, such as Germany, Brazil, and Mexico, have more than 50% of their population using the app.<sup>2,3</sup>

WhatsApp allows the user to send text messages, voice notes, files, localization, contacts, and voice and video calls for free since the user has access to the internet. In addition, the app has a strong end-to-end encryption enabled by default, based on Signal Protocol, which prevents third parties from having access to information exchanged between the users.<sup>4</sup> Thus, investigators can only access the data exchanged by WhatsApp users through the mobile phone seizure and later extraction and decoding of the app data. Nevertheless, it is common for criminals to erase suspicious contacts and messages to eliminate traces of criminal activity.

Several forensic tools support the extraction and decoding of WhatsApp data and, in some cases, can retrieve deleted messages from the SQLite database. However, some events related to the use of the WhatsApp, such as the deletion date of a chat, are not stored in the app's SQLite database and available mobile forensic tools cannot retrieve it. Moreover, sometimes only the evidence that a user talked to a certain contact or even had him on his contact list may already be enough to advance the criminal investigation. The event log files generated by WhatsApp store this type of information but are still unexplored by traditional mobile forensic tools.

WhatsApp log files store two types of information that mobile forensic tools do not explore. The first is data deleted by the user that the tools can no longer recover. The second is the information that WhatsApp does not save in its databases but stores in the events log file. Thus, this study developed a software tool to extract relevant information from the WhatsApp event log files to assist investigators in elucidating crimes.

The WhatsApp for Android™ saves very verbose event log files in the folder “/data/data/com.whatsapp/files/log.” These files store a detailed timeline of events related to the app, such as app activation date, connections to networks, contact synchronization, sent and received messages, deleted conversations, blocked contacts, exchange of encryption keys, user status, battery status, available memory on the device, and sound notifications, among many other types of events. These event log files can only be accessed with root permission on Android™ or by getting a physical image of the device memory. This fact, although it may seem like a problem, is an advantage, as it does not allow the ordinary user to delete them.

To find relevant data from these event log files for forensic use, experiments were conducted using an Android™ mobile device with root access and the WhatsApp version 2.18.191 installed, simulating various activities with the app and getting the corresponding events saved to the log file. Every event stored in the log file holds the date and time of occurrence, followed by information about the event. The content of the exchanged messages, for instance, is not stored, but the identification of the WhatsApp users involved in this activity is available, similar to many other cases.

The developed software, WAEventsLogParser, is free and can generate a detailed or grouped report containing the events found. Several events identified in this study are already configured in WAEventsLogParser and users can easily add more.

Aspects related to the use, distribution, and features of the software, as well as future development and strategies to identify the events, will be discussed in this presentation.

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2. *Number of Monthly Active Whatsapp Users Worldwide From April 2013 to December 2017 (in Millions)*. Statista, The Statistics Portal, January 2018, <https://www.statista.com/statistics/260819/number-of-monthly-active-WhatsApp-users/>.
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#### WhatsApp, Events Log File, Mobile Forensics



### C16 Breaking User Passwords in Android™ Devices Through Recovery Partition Substitution

*Pedro M.S. Eleuterio, MSc\*, Brazilian Federal Police, Campo Grande 79040010, BRAZIL*

**Learning Overview:** After attending this presentation, attendees will learn how to put the Android™ device in other boot methods to: (1) replace the original recovery partition of the Android™ device; (2) make a full internal memory backup without rooting the device; and (3) discover the smart phone user password, if it is a pattern, Personal Identification Number (PIN), or password. Attendees will also be able to understand where Android™ stores the password files and how to gain access to some password-protected Android™ devices to make a forensic analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing an additional method for experts to break user-defined passwords in some Android™ smart phones, allowing access to the data stored on the internal memory of a protected device. The presentation includes computer mobile forensics knowledge about Android™, including boot methods, password files, and security artifacts.

One of the main challenges in computer forensics is the analysis of smart phones, especially the device's internal memory. In the most cases, smart phone users protect their devices with Android™ native available patterns, PINs, or passwords. Thus, the computer forensics experts need to discover or bypass the lock to gain access to internal smart phone data. This study presents a new method to discover the user password in some Samsung™ smart phones with Android™ (up to version 6 (Marshmallow)) and with an external memory card slot.

Android™ has three types of initialization modes: Normal, Download (Fastboot) and Recovery, depending the keys pressed to power on the device. The strategy is to replace the original Recovery partition of the smart phone, using a custom recovery, known in some cases as Clock Work Mode (CWM) or TeamWin (TWRP). In some Samsung™ models, it is possible to replace the original Recovery Partition using Odin Software, initializing the smart phone in Download mode, and flashing the custom recovery. After replacing the recovery partition, the expert must initialize the smart phone in Recovery mode. The new custom Recovery often offers the option to backup the entire data of the internal memory of the smart phone to an external memory SDcard. Therefore, the forensic expert must use a blank memory card in the smart phone slot, which will store the full internal backup. In most cases, this backup also includes the protected user data, even without rooting the device. With the internal data of the smart phone, the forensic expert can discover the defined password, using forensic techniques, including a directed brute-force attack in the case of PIN/password protection.

If the user key is a pattern, the experts need to get the hexadecimal value stored in the "data/system/gesture.key" file, comparing it with a full pattern dictionary, which contains all pattern combinations, easily discovering the defined pattern. On the other hand, if the user key is a PIN or password, the expert needs to analyze some files, such as "data/system/password.key" (which stores the password hash), "data/system/device\_polices.xml" (which stores the password mask and size), and also find the password salt, which can be stored in "/data/data/com.android.providers.settings/databases/settings.db" or "data/system/locksettings.db-wal" files, depending on the Android™ version. With all that information, the forensic expert needs to brute-force attack the PIN/password, using a program such as Hashcat, directing the attack with a known mask observed within "device\_polices.xml" file. Hashcat is a free brute-force password attack program, which supports hundreds of password types, including MD5 and SHA-1, typically employed in Android™. In most cases, only a few minutes attack is necessary for Hashcat to discover the PIN/password, allowing the forensic expert to gain access to the smart phone and finally begin the forensic analysis of the device.

If recovering deleted files with data carving is not essential in the case, the experts do not need to obtain the user password since the full internal memory backup has all active data of the smart phone and, in some cases, this information is enough to discover the digital evidence contained on the smart phone.

Otherwise, there are known limitations of this strategy. For example, newer Android™ versions changed the way it stores the user password; some Samsung™ devices have Factory Reset Protection (FRP) or Secure Download Enabled, which prevent the partition from being overwritten; some Samsung™ models do not have a specific custom recovery; and the device will lose its warranty.

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**Mobile Forensics, Password Breaking, Android™**





### C17 Stego App Database (DB) and the Prevalence of Mobile Steganography

Jennifer Newman, PhD\*, Iowa State University, Ames, IA 50011; Li Lin, BS, Iowa State University, Ames, IA 50011; Wenhao Chen, BS, Iowa State University, Ames, IA 50011; Yong Guan, PhD, Iowa State University, Ames, IA 50011; Stephanie Reinders, BA, Iowa State University, Ames, IA 50011; Min Wu, PhD, University of Maryland, College Park, MD 20742

**Learning Overview:** After attending this presentation, attendees will be aware of the first mobile stego app database for image forensics and the current lack of software to detect the prevalence of stego use on mobile devices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing forensic criteria under which the dataset was designed and collected and will demonstrate that such a carefully curated and provenanced dataset can provide software developers with image data to benchmark tools they create on currently available stego data from mobile apps.

Tools for digital image analysis are advancing toward mainstream use in practical forensics. It is well known that reference datasets are necessary to help researchers develop and benchmark solutions for the variety and scale of data encountered by forensic practitioners.<sup>1</sup> For example, steganography has no known reference dataset to benchmark software that detects stego images occurring “in the wild.” While steganography is starting to consider more realistic scenarios, there is currently no known application that can test for steganography content originating from mobile device photographs created from stego apps on smart phones. Thus, the prevalence of steganography on mobile devices cannot be determined. This presentation introduces the first database consisting of mobile device photographs and stego images produced from stego apps on the phones, including a rich set of side information. The database contains images available to software developers to create steg detection programs—or steganalysis algorithms—that are more effective in detecting stego images produced by mobile apps. Once these tools are created and tested, it may be possible to begin investigating the prevalence of steganography use on mobile devices.

StegoAppDB, a steganography apps forensics image database, contains more than 810,000 innocent and stego images from ten different cell phone models (24 distinct devices) with detailed provenanced data including a wide range of International Organization of Standardization (ISO) speed and exposure settings, Exchangeable Image File Format (EXIF) data, stego Android™ Package Kits (APKs), message information, embedding rate, and other information. The data was collected according to a set of forensic criteria (authentication, representation, evaluation) and was free of copyright or privacy issues. The database is currently available to the public.

The acquisition procedure for original images will be discussed, including the camera app Cameraw, in both Android™ and iOS® systems.<sup>2</sup> The application allows researchers to gather multiple images per scene and saves each image simultaneously in both Digital Negative (DNG) and high-quality Joint Photographic Experts Group (JPEG) formats. From original images, stego images are created using five Android™ stego applications and one iPhone® stego application. Included are stego apps that write signatures and others that use random embedding methodologies. The extensive reverse engineering will be discussed, including source code modification and binary code instrumentation, the large amounts of innocent and stego image data that was generated for the benchmarking database. StegoAppDB provides cover-stego image pairs for each stego image, including applications that change input image dimensions, so that machine learning algorithms can be deployed for steganography detection.

Descriptive statistics of the database will be presented in addition to results of several experiments to substantiate the database’s investigatory nature. Applying three software programs to stego images from mobile apps—Stego Hunt, DC3 StegDetect, and Provos StegDetect—it will be shown that they are not adequate to detect stego images from modern stego apps.<sup>3-5</sup> The user interface, queries, and the download process of the website will be demonstrated. New data will continue to be added to the database on a monthly basis with the retained devices. While designed for steganography, possible uses of StegoAppDB to other digital image forensic topics will also be discussed.

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#### Steganography, Forensic Image Database, Steganalysis



### C18 Forensic Audio Analysis of Apple iPhone® Voice Memo Recordings

Catalin Grigoras, PHD\*, Denver, CO 80202; Jeff M. Smith, MS, National Center for Media Forensics - CU Denver, Denver, CO 80204

**Learning Overview:** The goal of this presentation is to disseminate preliminary findings related to the behavior of various Apple iPhone® voice memo recordings and different Advanced Audio Coding (AAC) decoding libraries as they relate to frequency and Modified Discrete Cosine Transform (MDCT) analyses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the results of a study that impacts laboratory performance and best practices in the handling and processing of audio and video containers that imbed AAC-encoded audio.

The Scientific Working Group on Digital Evidence (SWGDE) best practice guidelines for forensic authentication of digital audio recordings includes the MDCT and compression level analysis of the evidence recording.<sup>1</sup> This study is part of a larger research project regarding audio lossy compression artifacts analyses for forensic purposes.

This presentation will provide the results of a preliminary study on the discrimination between AAC lossy compression artifacts left by Apple iPhone® Voice Memo app and different other mobile phones and audio encoders and decoders. AAC lossy compressed signals are common in real forensic cases, can be produced with different mobile phones and software, and their forensic analysis and/or authentication can end up being crucial in the courtroom or other extrajudicial investigations.

This study reports on: (1) applications of MDCT to analyze the artifacts introduced by different recording devices, operating systems, and apps (e.g., Apple iPhone® Voice Memo, Samsung™ Voice Recorder; faad, fdk aac, FFmpeg, Nero, Adobe® Audition, iZotope RX Advanced, MATLAB, NCH, SuperSetup, etc.); (2) a fusion between Long Term Average Sorted Spectrum (LTASS), Compression Level (CL), and MDCT analyses of the lossy compression artifacts; (3) an automatic comparison method between the evidence signal and a database containing AAC, AC3, MP3, OGG, and WMA reference samples collected over a five-year span from more than 100 mobile phones, apps, OS, digital audio recorders, and core software applications; (4) an unbiased method to report the comparison results as Likelihood Ratios (LR) and error rates, and the automatic conversion of LRs on a verbal scale; and (5) the recording's length influence on the final results.

The suspect mobile phone, or original samples from it, and a database of reference recordings are necessary for a detailed forensic analysis, including the assessment of previous traces of lossy compression. Following previous research presenting the format and structure analysis, the preliminary results of this study indicate that the proposed framework can be used: (1) to verify Pulse-Code Modulation (PCM) signals and detect the presence or absence of MDCT artifacts left by previous lossy compression algorithms; (2) to discriminate between MDCT and up sampling artifacts; (3) to verify if an evidence AAC signal is consistent with an original or edited Apple iPhone® Voice Memo.M4A recording; and (4) to verify if a WAV PCM signal (e.g., an audio CD rip) is consistent with an original Apple iPhone® Voice Memo recording and discriminate between different iOS versions.<sup>2,3</sup>

Future presentations will present extended results and applications of the proposed framework on AAC, MP3, WMA verification and/or identification, and social media analysis. With these findings, the use of the proposed framework in forensic audio analyses together with other scientific validated methods is recommended.

#### Reference(s):

- <sup>1</sup>. SWGDE *Best Practices for Digital Audio Authentication*. Version: 1.2 (February 21, 2017).
- <sup>2</sup>. Grigoras C., Smith J.M. (2017) *Large Scale Test of Digital Audio File Structure and Format for Forensic Analysis*. 2017 AES International Conference on Audio Forensics, Arlington VA, USA.
- <sup>3</sup>. Smith, J., Lacey, D., Koenig, B., Grigoras, C. (2017) *Triage Approach for the Forensic Analysis of Apple iOS® Audio Files Recorded Using the "Voice Memos" App*. 2017 AES International Conference on Audio Forensics, Arlington VA, USA.

#### Audio, Apple®, Recordings



### C19 Android™ App Forensic Evidence Database

*Chao-Chun C. Cheng\**, Iowa State University, Ames, IA 50011; *Chen Shi, MS*, Iowa State University, Ames, IA 50011; *Brody Concannon*, Iowa State University, Ames, IA 50011; *Zhenqiang Gong, PHD*, Iowa State University, Ames, IA 50011; *Yong Guan, PhD*, Iowa State University, Ames, IA 50011

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**Learning Overview:** After attending this presentation, attendees will understand how to use this new Android™ Center for Statistics and Applications in Forensic Evidence-App Evidence Database (CSAFE-AED) in their casework investigation. This presentation will introduce the basics, challenges, and limitations of the current mobile device forensics, and demonstrate how to take advantage of the CSAFE-AED database and search/recover the possible evidence from the possible locations on and outside the mobile devices being investigated.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how this new Android™ CSAFE-AED can simplify and speed up the investigation procedures.

This project seeks to develop a set of automated Android™ app analysis tools (EviHunter) to discover all the possible evidence an Android™ app generates in the forms of files in the local storage, SQLite databases, or data sent to remote third party server(s). Authors from the CSAFE worked with researchers from the National Institute of Standards and Technology (NIST) to establish a dictionary-like Android™ app-evidence database (CSAFE-AED) that includes apps themselves (Android™ Application Package (APK) code, version, and meta data, (e.g., the number of downloads at the time the APK was collected)) and all possible evidential data (type, location(s), and evidence format/syntax) that each app can likely generate and store on the device or remote servers.

This presentation will introduce the basics, challenges, and limitations of the current mobile device forensics practice. A detailed explanation about the usefulness and availability of information about the potential evidence types and their locations on Android™ mobile device or remote servers (e.g., /data/data/com.app.foo/shared\_prefs/goos.xml → Visited URLs, Time) will also be provided. A demonstration will be given to explain how the usage of CSAFE-AED improves the overall mobile forensic analysis process via a set of carefully designed case works. In addition, this presentation will elaborate on the methodology and large-scale experimental evaluation of the approaches used to build CSAFE-AED. Overall, digital forensic investigators will learn how to take advantage of the CSAFE-AED database and search/recover potential evidence from the possible locations on and outside the mobile devices being investigated.

Based on the investigation of various app stores available globally, the number of various real-world apps has exceeded seven million so far. Commercial mobile device forensic tools, such as Cellebrite's Universal Forensic Extraction Device (UFED), support the profiles of approximately 6,000 apps and may not sufficiently support real-world mobile forensic case investigations. When digital forensic practitioners analyze mobile devices that had apps installed that are not currently supported by these tools, manual investigation has to be conducted over every single file extracted from the storage medium of the mobile device. Such manual casework may be error-prone and time-consuming. For example, a five-year-old Nexus® 7 tablet with 90 apps installed (both user and system space) can easily have approximately 20,000 files extracted from the device image. Performing manual forensic analysis on such a mobile device is arduous work, oftentimes simply infeasible to be accomplished within certain required time periods, which in turn may lead to more serious completeness and quality problems.

To tackle the challenges addressed above, a completely different approach was proposed and evaluated to provide much better coverage and precision guarantees. Both static and dynamic program analysis approaches were applied on analyzing/resolving evidentiary data, such as the file path and its corresponding evidence types. Currently, some existing work on privacy leakage problems are similar, but are not applicable for mobile app forensic analysis. To preserve the advantages (e.g., better time efficiency) provided by existing tools, a set of refined/improved automatic program analysis algorithms to analyze Android™ apps were implemented to create the CSAFE-AED. The CSAFE-AED also has the capability of handling new, updated versions of Android™ applications that are updated and published from time to time. The advantages of leveraging AED are: (1) mitigating false negatives caused by manual investigation; (2) improving the chances of identifying evidentiary data stored in specific format (e.g., custom encoding work); and (3) fast-tracking the evidentiary date of real-world apps.

The CSAFE-AED app forensic evidence database will be the first of its kind in terms of size (number of apps in the database) and accuracy/completeness of app analytical results. The CSAFE-AED database is expected to create fundamental and effective changes to current digital forensic practice, in particular mobile (Android™) device forensics.

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### Mobile Forensics, Android™ Apps, Digital Evidence



### C20 Go-Go Gadget, Smartwatch! An Investigation of Wearable Devices and Their Forensic Value

Nicole R. Odom, BS\*, Marshall University Forensic Science Center, Huntington, WV 25701; Jesse M. Lindmar, Virginia Department of Forensic Science, Richmond, VA 23219; Joshua L. Brunty, MS\*, Marshall University, Huntington, WV 25701; Catherine G. Rushton, EdD, Marshall University Forensic Science Program, Huntington, WV 25701

**Learning Overview:** After attending this presentation, attendees will better understand how smartwatch wearable devices with cellular network capability interact with companion mobile phones and where sensitive user data and forensic artifacts are stored, both through utilization as a standalone and as a connected device. This presentation will also provide a methodology for the forensically sound acquisition of data from a standalone wearable device.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering more insight into smartwatch wearable forensics, an area of research that is lacking. This presentation will provide an in-depth picture of not only what probative evidence each wearable may contain, but also the process of acquiring this data both directly from the smartwatch or through its companion mobile device, in order that analysts may most efficiently utilize their time and effort during investigations.

Smartwatch wearable devices allow users the ability to walk away from their mobile phone while remaining connected to the digital world. However, this freedom creates additional challenges for digital forensic investigators and analysts tasked with the examination, acquisition, identification, and analysis of probative data. Police departments are now finding that victims, suspects, and witnesses possess up to three smart devices each, as opposed to a singular mobile device.<sup>1</sup> This increase in smart device ownership leads to larger amounts of personal data being created, modified, and accessed. This personal data can be employed to establish causality in civil or criminal investigations. Features like standalone cellular network connectivity, automation through applications, and sensors that may result in persistent and invariable probative data, smartwatch wearable devices have the potential for an even broader scope and depth of information.<sup>2</sup> It has become imperative to understand how these devices operate and the challenges they present in order to provide insight into the implementation of data acquisition methodologies.

Very few studies have been performed on the acquisition of smartwatch data. Those that have been performed have utilized limited methods that are time-consuming, incomplete, or forensically unsound. One study has found that user data such as communication, app information, Personal Information Management (PIM) data, and calendar events are accessible through data acquired from various paired smartwatches. This indicates the forensic value of smartwatches is evident and warrants further exploration.<sup>3</sup> This preliminary research attempts to provide a better understanding of the sensitive user data and forensic artifacts stored directly on these wearable devices. User data observed is from devices connected with a companion mobile phone device or when utilized as a standalone device operating on a cellular network. The overall goal is to increase awareness of the valuable data these wearables are capable of storing and provide a methodology for how to access the data in future investigations.

For this research, the Samsung™ Gear S3 Frontier and Apple Watch® Series 3 were populated and examined. Two separate studies were conducted: data population in a connected state with a companion mobile phone device and data population in a standalone state operating on a cellular network connection. Following completion of both studies, two separate examinations were performed. The first involved the two mobile phone devices synced with the smartwatch wearables (i.e., the Samsung™ Galaxy S8 and Apple® iPhone® 6) to determine if any forensic artifacts were left from its respective smartwatch device and whether user data is stored when acting in a connected or standalone state. The second examination involved the smartwatch wearable devices and any identifiable data they may store that could be considered probative in a forensic investigation.

This presentation will provide the methodology implemented throughout the investigation, including data population and procedures for data acquisition, in an attempt to answer the following questions: what data can be found on the companion mobile phone device?; does the wearable device store data not found on the mobile phone?; and, in the case that a mobile phone is not present during forensic acquisitions, what probative data is available specifically on the wearable device? The results of this work will be presented, as well as general observations made throughout the investigation and any future directions or recommendations.

#### Reference(s):

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2. Rand Corporation. Digital Evidence and the U.S. Criminal Justice System: Identifying Technology and Other Needs to More Effectively Acquire and Utilize Digital Evidence. *RAND Research Reports*, 2015, <https://www.ncjrs.gov/pdffiles1/nij/grants/248770.pdf>.
3. Ibrahim Baggili et al. Watch What You Wear: Preliminary Forensic Analysis of Smart Watches. *2015 10<sup>th</sup> International Conference on Availability, Reliability and Security*, (August 2015): 303-311, <https://doi.org/10.1109/ARES.2015.39>.

#### Digital Forensics, Wearable Devices, Data Recovery



### C21 Using Reverse Projection and File Metadata Analysis to Calculate Speed in Recorded Digital Video

*Brandon Epstein, BS\*, New Brunswick Police Department, New Brunswick, NJ 08903; Marla E. Carroll, BS\*, Forensic Video & Audio Associates, Inc, Plantation, FL 33317*

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**Learning Overview:** After attending this presentation, attendees will understand a novel approach for determining vehicle speed in a fatal motor vehicle accident using frame timing metadata.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new approach to determining time difference between frames of digitally recorded video. This directly relates to how practitioners, the judiciary, and the public analyze video footage in critical incidents, such as police officer use of force and motor vehicle accidents.

On July 25, 2016, a fatal motor vehicle accident occurred in Edison Township, NJ. The accident was captured on a nearby restaurant's exterior video security camera system. At the time, no accident reconstruction was conducted. However, data was acquired from one vehicle's onboard Event Data Recorder (EDR). With no accident reconstruction completed, the data from the EDR could not be validated; an attempt was then made to determine the speed of that vehicle using the recorded security camera footage.

An initial review of the video footage was conducted and seven frames of video prior to impact contained enough newly encoded data to utilize in the examination. A reverse projection was conducted with those seven frames in order to place the vehicle at specific locations on the roadway to determine distances prior to impact. The digital video file metadata was then analyzed to determine specific intervals between each displayed frame. This metadata differs from information displayed in the recording system's settings (30 Frames Per Second (FPS)) and the originally reported frame rate of the video file (25 FPS). Additionally, the metadata may account for minute differences in time that each video frame is intended to be displayed at. The metadata also differs greatly from a common misconception that all recorded video is intended to be viewed at 30 FPS.

Using the identified distances and frame time difference, speed was calculated for the vehicle. A margin of error was also calculated based on an examiner's ability to accurately identify the location of a recorded image on the roadway. Comparison with the calculated speed as well as the margin of error was conducted against the EDR reported speed data. It was found that the speed calculated from the recorded video was validated by corresponding EDR data speeds.

This approach to calculating speed from recorded video can be applied to other fatal accident investigations as well as any video footage where object speed is in question. With the prevalence of police body-worn cameras and bystander video in police use-of-force incidents, these questions are certain to become more popular. It would greatly benefit the forensic science community and the public to have a scientific basis for determining exact timing and speed of actions within digitally recorded video.

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#### Digital Evidence, Video Analysis, Crash Reconstruction



### C22 Smarthome Internet of Things (IoT) Traces: Forensic Potential and Challenges

*Eoghan Casey, PhD\*, University of Lausanne, Lausanne, Vaud, SWITZERLAND; Joshua I. James, PhD, Hallym University, Korea, Gangwon 24252, SOUTH KOREA; Francesco Servida\*, UNIL, Lausanne 1005, SWITZERLAND*

**Learning Overview:** After attending this presentation, attendees will better understand traces from IoT devices in smarthomes, how they can be useful in any investigation, and the challenges associated with evaluating these digital traces. This presentation has three objectives: (1) increase familiarity with traces from various IoT devices in a smarthome; (2) demonstrate how traces from IoT devices in a smarthome can be useful for investigative and forensic purposes; and (3) discuss evaluation challenges associated with traces from IoT devices in a smarthome.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing traces from IoT devices in smarthomes, explaining the value of traces for any investigation, and the challenges associated with collection and evaluation of such traces when investigating criminal offenses.

IoT devices that provide home entertainment, comfort, convenience, and security can also contain vulnerabilities and generated traces. These digital traces can be useful for investigative and forensic purposes in any type of offense, including violent crime. At the same time, these traces can present evaluation challenges for forensic scientists and can create privacy risks for people in their homes.

Research and testing of various IoT devices was conducted in coordination with the Digital Forensic Research Workshop (DFRWS) IoT Forensic Challenges. These activities implemented various methods for obtaining traces from IoT devices, led to a deeper understanding of the traces generated by these devices, and led to tool development for specific types of traces. The simulated case scenarios focused on violent crime in order to connect digital traces with physical world offenses.

A combination of commercial, open-source, and bespoke methods were used to extract and analyze the traces. Traces were obtained from IoT devices themselves, from smart hubs, and from associated smart phone apps. Some information was obtained from network traffic and cloud-connected servers, but many transient or cloud traces were treated as out of scope in order to concentrate attention and activities on the physical location of a crime. Specialized forensic capabilities emerging from this work have been made available as open source.

Results of the coordinated research and testing are presented to highlight the potential value of such digital traces in any case, including violent crime investigation. In addition, the challenges associated with IoT traces are presented. Specifically, the challenges associated with preservation and analysis of these traces will be presented, and the difficulties in evaluation of forensic findings will be discussed. The need for future work is emphasized in order to keep pace with the rapid development of new smarthome IoT devices. Privacy and cybersecurity issues are raised in general terms to increase awareness of the risks associated with such devices.

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**Digital Forensic Science, Smarthome Forensic Analysis, IoT Trace**



### C23 Diving Into Blockchains Contents: The Bitcoin Snapshot

*Oliver Giudice, PhD\*, Roma 00044, ITALY*

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**Learning Overview:** The goal of this presentation is to present a method for blockchain analysis with the goal of discovering non-transactional information, such as image files, text files, etc. Attendees will be introduced to the basics of blockchain technologies and Application Programming Interfaces (APIs) of the bitcoin protocol to understand end-user capabilities. Finally, blockchain diving techniques will be presented to assist attendees in creating automatic tools for blockchain analysis and content retrieval.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how blockchain technology is a novel field in which new crimes are emerging (illicit content sharing, Intellectual Property (IP) violations, etc.), thus highlighting the need for new methods, techniques, and expertise.

Blockchain technology in recent years has been defined as the new generation of internet, or rather a type of internet of transactions. There is no definition of blockchain that is universal, but it is a concept that has several more or less valid interpretations.

Blockchain technology actually belongs to an even broader concept, Distributor Ledger Technology (DLT). DLTs are distributed databases, meaning they can be updated, managed, controlled, and coordinated not only at the central level, but in a distributed way, by all the actors participating in the DLT. Each user can manage a node, but each transaction must first be approved by most participants in the network. DLTs use independent computers (called nodes) to record, share, and synchronize transactions in the corresponding ledgers (master books). The use of new technologies could certainly have a major impact on the world economy due to many elements, such as security, the speed of transmission of transactions, and anonymity. DLT applications could very quickly replace many tasks that are still being performed manually today.

Bitcoin is an implementation of blockchain technology, which is the most known and used today. However, while blockchains can be a great technology for transactions of value, they do not contain data related only to transactions. However, they can contain other information, such as text files or images.

The problem arises when this data is illegal material; anyone who memorized and shared the material could be accused of possession of that illegal information. Thus, the need for a blockchain analytics tool arises to detect non-transactional content diving into a large-scale quantity of data.

Writing non-transactional information is very easy; specifically in bitcoin, it is possible to insert in the place of the address any string, be it an address, a text, or a link. In doing so, of course, there is a waste of money, but irrelevant sums could be inserted. Once the desired string has been inserted, the shared material is visible and can be downloaded from every node present in the network.

As an example, it is possible to look at the third block of the bitcoin blockchain and obtain as a result a set of characters that make up the face of a man.

Techniques will be presented that are able to automatically detect non-transactional information in the bitcoin blockchain by means of address parsing, transactions content analysis, and analysis of the OP\_RETURN field.

The simple technique that will be described in this presentation is the ability to identify more than 1,500 non-transactional contents present on the bitcoin blockchain until December 2017. All those contents can be divided into the following types: source code files, HTML files, image files, and text files.

Finally, issues related to illegal material on blockchains will be discussed with respect to contents extracted from the bitcoin blockchain.

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#### **Bitcoin Forensics, Bitcoin Content Analysis, Illicit Media Sharing**



### C24 Face Morphing Detection

*Ilias Batskos, BSc\*, NFI and University of Amsterdam, Den Haag 2497GB, NETHERLANDS; Andrea Macarulla, MSc\*, NFI and University of Amsterdam, Den Haag 2497GB, NETHERLANDS; Zeno J. Geradts, PhD\*, Netherlands Forensic Institute, Den Haag, SH 2497 GB, NETHERLANDS*

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**Learning Overview:** After attending this presentation, attendees will understand: (1) the basic principles of this identity-sharing scheme, (2) the state-of-the-art detection methods and their vulnerabilities, (3) a novel detection method, and (4) measures that render the scheme irrelevant.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness regarding the risks of current security practices in passport issuance and automatic border controls by pointing out the vulnerabilities and will foster progress by proposing a novel detection scheme.

The goal of this research is to develop a method for detecting face morphing, which is the procedure of creating a novel photograph by blending, usually with equal contribution percentages, two photographs of two different persons using computer software, either manually or automatically. The created photograph is then printed by the applicant and sent to the issuing authorities. If the photograph passes successful human inspection, it is scanned and uploaded to the document's microchip. Depending on the quality of the end result, the morph can bypass both human and machine verification controls at the time of issuing and at the Automatic Border Control verification stages of electronic Machine Readable Travel Documents (MRTD). That means that the e-MRTD can be successfully used by both morph contributors, one being the criminal and the other the accomplice.

The method is not based on detecting micro-traces on pixel level and thus should not be affected by the inevitable loss of information due to the print and scan process or by sophisticated concealment of morphing traces, which is the Achilles heel of conventional detection methods. Instead, the probe photo is morphed with the e-Pass photo. Face encodings are extracted from each of the three photographs (probe, e-Pass, and morph) and similarity scores are calculated between the probe and the e-Pass (d0), the morph and the probe (d1), and between the morph and the e-Pass (d2). These three distances comprise the characteristic vector for each specific case, which is then classified by a classifier trained with vectors of genuine and criminal scenarios based on the hypothesis that criminal vectors include biometric information from two different individuals and will thus be different from genuine vectors, which include biometric information from a single individual.

The training set consists of 20 genuine and 20 criminal cases. One genuine and one criminal case were misclassified. The testing set consists of 59 genuine and 48 criminal cases, different from those of the training set. A couple of genuine and one criminal case were misclassified. The classifier achieved a True Positive Rate (TPR) of 0.966 and a False Positive Rate (FPR) of 0.0208. TPR is the proportion of actual positives (genuine scenarios) that are correctly identified as such, and FPR is the proportion of actual negatives (criminal scenarios) that are falsely identified as positives.

The promising experimental classification results show that the 3D vector could be used as an additional security layer next to the conventional detection methods, assisting a correct decision.

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### Biometrics, Passports, Manipulation



## C25 Analysis of “Deepfakes” Creation and Detection: Video and Image Fabrication Using Deep Learning

Jeff M. Smith, MS\*, National Center for Media Forensics, CU Denver, Denver, CO 80204; Catalin Grigoras, PhD, Denver, CO 80202

**Learning Overview:** After attending this presentation, attendees will have a better understanding of how deep learning can be used to fabricate faces in images and videos and the challenge facing forensic examiners in the analysis of these videos.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by shedding light on how “deepfakes” face swaps are created, including the software and hardware requirements, and help in understanding the real threats facing examiners in forensics and intelligence.

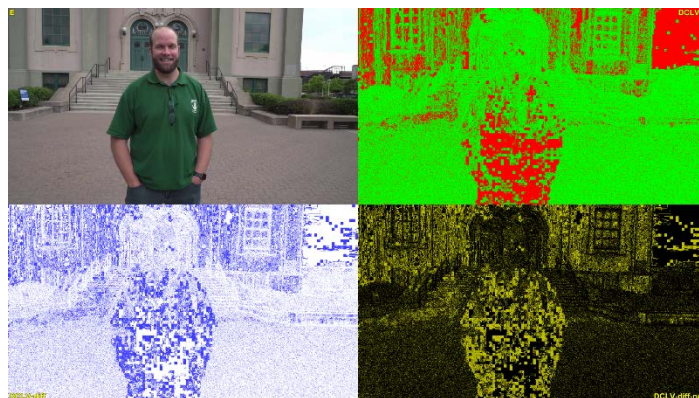
Artificial Intelligence (AI) presents a boon of technological capabilities in many areas that will affect lives for years to come. With today’s research in driverless cars, big data, multimedia authentication, and facial recognition technology, it is clear that AI and machine learning will be some of the underlying technologies relied upon in the future. The recent trend to use deep learning to train models for face swapping has made an impact on how the public perceive the future of media, the news, and the entertainment industry. In late 2017, reddit® Inc. website user “deepfakes” posted well-known celebrities’ faces swapped onto adult film content using these machine learning techniques. Since then, non-consensual content of this nature has been banned from the reddit® website and many other well-known sites, but Safe For Work (SFW) image and video deepfakes have been very popular on the internet since then. This has led to a shift in the public perception of the trustworthiness of media and the potential spread of fake news in the future becoming a real threat. Deepfakes viral videos demonstrate to the general public that a technology can be used to create convincing false content videos. Fake videos of one person saying what is not true content and not actually what the original person had said with the appearance of accurate facial expressions and mouth movements is a prospect that has the public concerned.

During this presentation, many examples will be shown. With this research, the realities of producing deepfake face swaps (Figure 1) using current technology will be discussed, including the Python machine learning libraries and Graphics Processing Unit (GPU) hardware processing that make it possible. Limitations will be explored as well as the potential advantages of using deep learning for video content creation over traditional manual or automated copy/paste methods. Finally, a discussion of the forensic detection of deepfakes fabricated videos in a forensic setting (Figure 2) will be covered.

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**Figure 1:** Frame of video face swapped (center) using deep learning given the source face (right) swapped onto the original face and body (left).



**Figure 2:** Video authenticity plots of a deepfakes face swapped video frame.



### C26 Non-Obvious Links in Online Frauds: Modeling and Comparison of Digital Traces, Context, and Actions

Timothy Bollé\*, School of Criminal Justice, University of Lausanne, Lausanne, Vaud 1015, SWITZERLAND; Eoghan Casey, PhD, University of Lausanne, Lausanne, Vaud, SWITZERLAND

**Learning Overview:** After attending this presentation, attendees will better understand how to produce crime intelligence from real-world cases by combining techniques from forensic science and computer science and detecting non-obvious links and patterns. Attendees will also have a better understanding of the analysis of the detected links and patterns and of the resulting interpretation and decision-making processes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping investigators and analysts to have a more complete vision of the available information, which will reduce the risk of missed opportunities caused by data silos and linkage blindness.

The goal of this research is to explore new and existing techniques and methods from forensic science, crime intelligence, and computer science to find non-exact or non-obvious similarities between online frauds. Perpetrators can easily switch to different online accounts or platforms, effectively changing their digital identity and the traces of their activities. As a result, exact comparison of digital traces is a limitation for link discovery in online fraud investigations. To overcome this limitation and to avoid linkage blindness, it is necessary to use near similarity comparison of distinctive characteristics of online frauds, including digital traces, context (spatio-temporal information), and actions taken by authors (*modus operandi*).

In order to correlate these distinctive characteristics, it is necessary to centralize the information and to structure it. This structure should allow the detection of links and patterns between entities. The main challenge here is the representation of actions and their context. The Cyber-investigation Analysis Standard Expression (CASE) will be tested to evaluate if it fits those objectives.<sup>1</sup>

In this work, a dataset of real-world online frauds will be used to test the validity of the proposed approach. After a general description of the dataset, algorithms to compute near similarity between digital traces are used to discover new links between cases. Furthermore, some of the cases will be studied more closely and represented using the CASE standard. The objective here is to model the actions taken by the authors, which allows their comparison, and then the detection and the analysis of patterns of actions.

An integral part of this work is to study the decision-making process of evaluating the links found using near similarity computations. An aspect of this evaluation could be to combine multiple information to look at the context of a given link and to compare the differences between nearly similar cases. It will help the investigator or the analyst to analyze the detected link and make suitable decisions.

Such crime intelligence approaches allow the centralization of information regarding multiple online fraud cases. It will help the investigators and the analysts to have a more complete vision of the available information, which will reduce the risk of missed opportunities caused by data silos and linkage blindness. Finding links and repetition between multiple cases is useful to have a better understanding of the various phenomenon in online frauds. This knowledge can be used in future investigations to obtain known useful traces and to apply efficient investigation methods and techniques. It can also be used to raise awareness about existing frauds. Linking cases committed by a potential same group of authors also allows them to be considered as one case, which increases the quantity of information about the group and is easier to handle for prosecutors.

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1. Casey, Eoghan, Sean Barnum, Ryan Griffith, Jonathan Snyder, Harm Van Beek, and Alex Nelson. Advancing Coordinated Cyber-Investigations and Tool Interoperability Using a Community Developed Specification Language. *Digital Investigation*. 22 (September 1, 2017): 14–45. <https://doi.org/10.1016/j.diin.2017.08.002>.

#### Forensic Intelligence, Near Similarity Computations, Online Frauds



### C27 Proving Database Tampering Through In-Memory Object Reconstruction

Aisha Ali-Gombe, PhD\*, Towson University, Towson, MD 21252; Sneha Sudhakaran, MTech, Louisiana State University, Baton Rouge, LA 70808; Andrew Case, MS, Louisiana State University, Baton Rouge, LA 70808; Golden G. Richard III, PhD, Louisiana State University, Baton Rouge, LA 70808

**Learning Overview:** After attending this presentation, attendees will gain an understanding of in-memory object recovery from userland process address spaces on Android™ devices and how the retrieved objects and their metadata can be leveraged to reconstruct database queries in system-wide content providers and prove database access and manipulation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how memory forensics can be used to generate causal relationships between applications and native system database accesses. Reconstructions with real applications of allocated objects from process runtime memory will be demonstrated and their metadata will be used to trace database activities. This approach will illustrate how vital this detection process is in proving attribution on a multi-app platform, such as Android™.

The Android™ operating system stores system-wide sensitive user data, such as text messages, call logs, and calendars, in SQLite databases, which are accessible through a management service called a native content provider. Each provider is associated with a single SQLite database file. To access any SQLite data object, an application makes a Create, Read, Update, and Delete (CRUD) request to the provider using a content resolver object. Each CRUD function corresponds to one of the SQL data manipulation queries (insert, update, delete, and select).<sup>1</sup> The most important object in a CRUD function is the Universal Resource Identifier (URI), which tells the resolver which provider to contact and collectively with the remaining parameters informs the provider about which query should be performed.

In this research work, the objective was to recover important objects allocated by user processes at runtime, then use their metadata to reconstruct events. On the new Android™ Runtime (ART), dynamic objects are allocated using the RosAlloc allocator. The RosAlloc provides an efficient method of allocating sequential memory space to objects of the same size.<sup>2</sup> Every allocated object is created based on its class specification and contains a pointer within its memory space to its definition. Also, it has an associating lock that holds the ArtMethod for the object. To recover database activities, individual threads in the process runtime are identified, then all the allocated runs per thread and their associated non-free slots are recovered. Based on the object class definition, URI class objects are enumerated and parsed. The metadata of those objects will be traced to identify the “locking” CRUD function. Finally, the resulting data will then be correlated with a low-level parcel recovered from registered system-wide Inter-Process Communication (IPC) threads.

#### Reference(s):

1. Ali-Gombe A., Richard G.G. III, Ahmed I., Roussev V. Don't Touch that Column: Portable, Fine-Grained Access Control for Android's™ Native Content Providers. *Proceedings of the 9<sup>th</sup> ACM Conference on Security & Privacy in Wireless and Mobile Networks*; July 2016, Darmstadt, Germany, (pp. 79-90). ACM.
2. Bhatia R., Saltaformaggio B., Yang S.J., Ali-Gombe A., Zhang X., Xu D., Richard G.G. III. Tipped Off by Your Memory Allocator: Device-Wide User Activity Sequencing from Android™ Memory Images. *Proceedings of the 2018 Network and Distributed System Security Symposium (NDSS 2018)*; February 2018, San Diego, CA.

**Android™, Memory, SQLite**

## C28 Hands-On Digital Image Authentication Techniques

Oliver Giudice, PhD\*, Roma 00044, ITALY; Antonino Paratore, MS, ICTLab S.R.l. Spinoff of Università di Catania, Catania 95125, ITALY; Sebastiano Battiato, PhD\*, Università di Catania, Catania 95125, ITALY; Luca Guarnera, MS, Università di Catania, Catania 95125, ITALY

**Learning Overview:** After attending this presentation, attendees will be familiar with various techniques for image authentication based on double quantization detection analysis and first quantization step estimation of a digital image. Due to the widespread digital image forensics technique, this presentation will attempt to make a uniformness among all the existing solutions by presenting a simple but schematic view. All techniques will be presented organized in categories and the pros and cons exposed with regard to practical application scenarios.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of a wide range of solutions and the forensics community will be able to select the most accurate technique for a specific investigation case.

The widespread diffusion of digital image technology over the past decades has increased the interest in image integrity verification, thus becoming one of the main goals in multimedia forensics. Understanding if a certain image was previously compressed, together with any information related to past processing, is an extremely powerful tool for forensic examinations. Some of the retrievable information, such as the first quantization step used by JPEG algorithms at image acquisition time, represents one of the fundamental parameters for both image tampering detection and source camera identification. In this presentation, after presenting the fundamentals of JPEG compression and the traces left on digital images, the most significant state-of-the-art techniques for integrity verification by making use of first quantization step estimation will be illustrated and critically compared.

Every day millions of people safely store and share many moments of their lives through social networks. All that data is digitally stored in multimedia content through their digital devices, such as mobile smart phones. Thus, forensic investigations can take advantage of that data through evidence collection of the images and videos and reconstruct a specific crime event. To check the originality of these digital clues, a new domain was recently created called image forensics, whose goal is to leverage the knowledge of image processing to answer questions that arise in an investigative scenario.<sup>1,2</sup>

In this complex environment, since JPEG has emerged as the most popular compression standard for digital images, data related to the image processing pipeline have been deeply analyzed by research communities in order to identify the traces left by the compression algorithm on an image.<sup>3-5</sup> To this end, one part of the algorithm most examined was the Discrete Cosine Transform (DCT), a mathematical tool applied on images to shift from spatial domain to a domain of frequency.

Throughout the years, research papers that provided an overview on state-of-the-art methods in image forensics did not perform an in-depth exploration of each aspect of DCT analysis, probably due to the difficulty of joining insights from several methods.<sup>6-16</sup> The purpose of this presentation is to fill this gap, which has never been covered as a stand-alone topic. The most important methods that try to model the behavior of DCT coefficients when a JPEG image is decompressed after the shoot, edited, and then compressed again, or stored in uncompressed format, will be described with pros, cons, and application scenarios.

### Reference(s):

1. B. Zhu Bin, M.D. Swanson, and T.A.H. When Seeing Isn't Believing (Multimedia Authentication Technologies). *IEEE Signal Processing Magazine*, vol. 21, no. 2, pp. 40–49, 2004.
2. <https://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/oct2005/standards/>.
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7. E. Kee, M.K. Johnson, and F.H. Digital Image Authentication From JPEG Headers. *IEEE Transactions on Information Forensics and Security*, vol. 6, no. 3, pp. 1066–1075, 2011.
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### Double Quantization, Multimedia Forensics, Image Authentication

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C29 WITHDRAWN



### C30 A New Botnet Command and Control Mechanism Using the Ethereum Platform

*Joshua Ralls, Eastern Kentucky University, Richmond, KY 40475; Shuangteng Zhang, PhD\*, Eastern Kentucky University, Richmond, KY 40475*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of botnets, Command and Control (C&C) servers, and blockchain technology, as well as the possible use of the Ethereum platform to create botnet C&C server to automatically launch cyberattacks, such as ransomware attacks, and the corresponding possible countermeasures against these attacks.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a possible new cyber attacking approach that takes advantage of the blockchain technology, which can make the attacks more difficult to track and shut down, and through discussing the corresponding defending and investigation strategies on these types of attacks.

Botnets are a group of compromised internet-connected machines remotely controlled by the botmaster that serves as the C&C server to send commands to and receive information from the bots, the individual machines consisting of the botnet. Using botmaster and botnets, the malicious users can launch Distributed Denial-of-Service (DDoS) attacks, spread malware such as ransomware, distribute spam, steal data, and more. To successfully take down or disrupt the operations of the botmaster and botnet, bots are usually analyzed to trace and discover the botmaster and the bot network.

The current botnet attacking relies on the botmaster with a centralized management. Therefore, the botmaster becomes the weakest point of botnet infrastructure. Once the botmaster is discovered, many takedown techniques can be used to counteract the operations between botmaster and the bots. To avoid the botmaster from being revealed, many techniques such as HTTP botnets, domain flux, and peer-to-peer botnets are used by malicious users. However, even with these techniques, the botmaster and botnets are still traceable and can be taken down or disrupted.

Blockchain technology provides a new way of storing information in a distributed ledger that allows a reliable and secure sharing of and access to the same information. This technology can be applied to numerous applications for good purposes. However, because of its decentralized management of information, blockchain technology may possibly be used by the malicious users to build a botmaster and botnet infrastructure and make it, if not impossible, at least difficult to trace and take down.

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#### Botnets, Command & Control Server, Ethereum



### C31 Rapid Differential Forensic Acquisition Using Limited Resource Computing

Mark D. Guido, MS\*, The MITRE Corporation, Mclean, VA 22102; Vikram Harichandran, MS, Fairfax, VA 22031; Glenn A. Melton, BS, The MITRE Corporation, Mclean, VA 22102

**Learning Overview:** The goal of this presentation is to describe further development of a previously presented topic, Rapid Differential Forensic Imaging, to be applied on an appliance-based computer with limited computing resources.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a reference architecture addressing a limited resource computing environment typically found during forensic investigations.

In 2016, an automated differential forensic acquisition technique and algorithm that uses baseline datasets and hash comparisons to limit the amount of data sent from a mobile device to an acquisition endpoint was introduced. It was possible to produce forensically validated bit-for-bit copies of device storage in significantly reduced amounts of time compared to common commercial products. For example, using this technique, an average initial imaging rate of less than seven minutes per device for a corpus of actively used, real-world 16 GB Samsung™ Galaxy smart phones was successfully achieved. If the need arose for further acquisitions of the same device, then the timeframe for those acquisitions would be significantly quicker due to the information gained and utilized from the initial acquisition. A reference implementation of the algorithm and architecture was developed, and the technology has been successfully transferred to customers and industry.<sup>1</sup>

Now, a new implementation of the software running on a tiny form-factor limited-resource computing device is introduced. The device chosen was a Raspberry PI3 Model B, although it is thought that most low-resource computing platforms would be able to identify and host this described reference implementation. This device is considered an appliance-based form of the original algorithm and software, where power considerations, processing power, persistent storage, and volatile memory all needed to be refactored to make a viable solution. This addresses the use case of a limited resource environment typically found when forensic acquisition is required in the field. The process for forensic acquisition had to change when no longer provided the luxury of always-on connectivity between the front-end client and back-end server and storage was no longer provided. It was also necessary to monitor the consumption of power, processing, storage, and memory so as not to enter an exhausted state.

Details of the architectural changes, resource considerations, and in-field testing are documented herein. The changes made for the limited computing reference implementation can also address other use cases in which one or more resources may be reduced or limited, as is the case for low bandwidth, cross-geographic communications of forensic images. Those use cases will also be briefly addressed.

**Reference(s):**

<sup>1</sup>. Guido, M., J. Buttner, and J. Grover. Rapid Differential Forensic Imaging of Mobile Devices. *Digital Investigation*. 18 (2016): S46-S54.

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**Differential Forensics, Acquisition, Appliance**



### C32 Reevaluating the Mobile Forensic Acquisition Levels

*Troy Lawrence, BBA\*, Fort Worth Police Department, Fort Worth, TX 76107; Umit Karabiyik, PhD\*, Purdue University, West Lafayette, IN 47907*

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**Learning Overview:** After attending this presentation, attendees will be able to clarify the confusion in mobile device acquisition methods as well as understand the current acquisition methods and their impact on the devices with respect to rapidly changing technology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an updated common framework for mobile device acquisition in digital forensics investigations.

Mobile Device Forensics (also known as Mobile Phone Forensics) is a subdiscipline of Digital Forensics, much like Computer Forensics, Audio Forensics, and Forensic Video. This subdiscipline is relatively new and did not begin to gain popularity until just prior to the invention of the iPhone® in 2007. Forensic examiners used manufacturer-developed tools designed to backup user data or were forced to photograph data as it was displayed on the screen prior to the development of mobile device forensic tools. It was during this time frame that a schema was developed to categorize the types of forensic tool capabilities. In 2008, Sam Brothers, a mobile forensic examiner working for the United States Customs and Border Protection, first articulated the current classifications of mobile device forensic tools.<sup>1</sup> The five levels of classification for forensic tools include: (1) manual extraction, (2) logical extraction, (3) hex dump/JTAG, (4) chip-off, and (5) micro read.

The pyramid diagram was later incorporated into the National Institute of Standards and Technology's (NIST's) SP800-101r1 and is referenced in many books, documents, and articles describing not only the forensic tool classifications but also the elevated levels of data acquisition.<sup>2</sup> For years, Mobile Device Forensic examiners have been well-served using Brothers' popular pyramid listing five levels of data acquisition from mobile devices. However, processing methods have evolved over the ten years since the diagram was introduced. There is confusion regarding which level of extraction applies to a process as new techniques are continually being developed. A re-evaluation of the Mobile Forensic acquisition levels and the associated tool capability pyramid graphic is needed to reflect the current methods of mobile device acquisition and their impact on the devices. Some of the more advanced levels of acquisition may require extensive training and years of practice to become proficient in their use. Others may require no specific skills other than operating a camera to photograph a screen. Regardless, the levels of acquisition need to be properly identified based upon the type of data collected, how it is collected, and whether it may damage the device.

The presenters will provide clarity to the extraction process and propose an update to Sam Brothers' forensic tool categories of acquisition as well as adequately describe each option currently available. Although it may be necessary in the future to update these processes as new acquisition techniques become available, it is believed that the updated framework will serve the digital forensics community for better understanding of the acquisition methods with respect to the state-of-the-art mobile device investigations.

**Reference(s):**

- <sup>1</sup> Samuel Brothers. *Cell Phone Forensic Tool Classification Pyramid*. Presented at Mobile Forensics World 2008, Breakout Presentation, Chicago, IL, May 2008.
- <sup>2</sup> Wayne Jansen, Rick Ayers, Sam Brothers. Guidelines on Mobile Device Forensics. *NIST Special Publication* (2014): 800-101.

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### Digital Forensics, Mobile Devices, Acquisition Levels





### C33 iPhone® Video Metadata: What Can It Teach Us About a Recordings History?

*James Zjalic, MSc\*, Birmingham, England B62 0EZ, UNITED KINGDOM; Jeff M. Smith, MS, National Center for Media Forensics, CU Denver, Denver, CO 80204; Cole Whitecotton, MS, UC Denver, Englewood, CO 80110; Catalin Grigoras, PhD, Denver, CO 80202*

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**Learning Overview:** After attending this presentation, attendees will understand how video recordings made with Apple® iPhone® devices change based on both the method used to download the files and whether they have been edited within an Apple® device. These changes can provide vital information to inform us as to the recording's history.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating why all iPhone® multimedia should be considered with caution and by providing a methodology as to how to determine the history of a recording. This will specifically aid those who perform video authentications and, in a broader sense, reveal why exhibits should not be taken at face value as authentic without a consideration as to the history of the exhibit.

The need for research into this area became evident during a forensic examination of an Apple® iPhone® video recording that was found to have iOS® metadata that didn't relate to the phone it was extracted from. This led to the present research into iOS® video, from which previous work into the iOS® Voice Memos App is built upon.<sup>1</sup> With this research framework, video is captured in all available manipulation and sharing scenarios in order to examine the various states that video data can take in these situations.

Video imagery captured using mobile phones can form a large area of work conducted by forensic media experts and suffers from two key issues in terms of reliability. The first is that the data can easily be transmitted via text message or email and saved to a new device, masking its origin. The second is that the mobile phones themselves have features that allow for the editing of the data. Combining these two factors can complicate examinations, as the possibility can exist that the data was edited on another device, sent via email to the seized device, and saved.

Understanding the provenance of multimedia is essential for the chain of the custody and, specifically, to authentication examinations. Without knowing where an image, audio, or video recording first originated, the possibility can exist that the evidence will later be deemed inadmissible and any further work conducted (such as enhancements) will become worthless. In the case of this presentation, the analogous links in the chain of custody are the transmissions of emails between phones and computers. Research has shown that there is the potential that some of these links may be missed, and edited multimedia can easily go undetected and accepted at face value, even after authentication analysis. Attendees will come to understand why all multimedia data obtained from iPhones® should be treated with caution with regard to its provenance and how to best determine the history of the version provided.

The research that informs this presentation is part of a larger research project that will also see the investigation of the changes that occur in audio, images, and video when edited and sent via differing transmission channels, such as email, text message, and iMessage®.

#### Reference(s):

- <sup>1</sup> J. Smith, D. Lacey, B. Koenig, and C. Grigoras. Triage Approach for the Forensic Analysis of Apple iOS® Audio Files Recorded Using the "Voice Memos" App. In: *Audio Engineering Society Conference: 2017 AES International Conference on Audio Forensics*, 2017.
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**Video Authentication, Video Analysis, iPhone® Analysis**



### C34 Instagram™ Forensic Artifacts on Windows® Systems

*Carol S. Smith, BS, Kentucky State Police, Electronic Crime Branch, Frankfort, KY 40601; Shuangteng Zhang, PhD\*, Eastern Kentucky University, Richmond, KY 40475*

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**Learning Overview:** After attending this presentation, attendees will understand the features of the Instagram™ web app, will know what (and where) possible forensic artifacts may be left on the Windows® systems by the Instagram™ users, and how those artifacts may be retrieved.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing knowledge regarding the Instagram™ forensic artifacts on Windows® systems. This knowledge will help digital forensic investigators in their investigations involving the Instagram™ app.

Social networking sites and applications have changed the way people share information and communicate with each other. With the popularity and intensive use of these sites and apps, more and more investigations of the criminal and civil cases involve the forensic analysis of user profiles and activities created in the social networking sites and apps. However, the existence of the many different social networking sites and apps, as well as the fact that they run on various platforms that are changed and updated (usually within a short period of time), has created a great challenge for those investigations. With this challenge, it is important for digital forensic investigators to have updated knowledge regarding what and how the forensic artifacts existing in the social networking sites and apps can be discovered and retrieved.

Instagram™ is one of the most popular social networking apps used among young people. It can be used to share photos and videos through users' smart phones. When an Instagram™ account is created for a user, a user profile and a news feed are also created and associated with the account. The photos and videos the users post are displayed on their profiles and can be seen and commented on by their followers in their own news feed. Therefore, when a civil or criminal case involves the Instagram™ app, accessing the user's profile and analyzing his or her Instagram™ activities will provide valuable information for the investigation. However, today's smart phones are getting more and more secure and the information on them has become more and more difficult to retrieve. This has presented a challenge for an investigation involving the Instagram™ app. Fortunately, the account of each Instagram™ user can also be accessed through Instagram.com using a web browser. This provides an alternate way for retrieving the profile information of the Instagram™ users as well as their Instagram™ activities.

This presentation will provide an overview of Instagram's™ features in both the mobile and web versions. Focusing on Instagram's™ web app, this presentation will discuss what and how forensic artifacts, such as login information, profile information, and user activities, can be retrieved. This presentation will also discuss the retrieval of possible artifacts in the third-party apps that are used to upload photos to user's Instagram™ account.

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#### Instagram, Forensic Artifacts, Windows Systems



### C35 Fuzzing Memory Forensics Frameworks

*Arian Dokht Shahmirza, BSc\*, Louisiana State University, Baton Rouge, LA 70808; Aisha Ali-Gombe, PhD, Towson University, Towson, MD 21252; Andrew Case, MS, Louisiana State University, Baton Rouge, LA; Golden G. Richard III, PhD, Louisiana State University, Baton Rouge, LA 70808*

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**Learning Overview:** Fuzz testing memory forensics frameworks can reveal important vulnerabilities that are crucial in the effort to create robust forensics tools. After attending this presentation, attendees will be familiar with fuzzing techniques that are applicable to testing memory forensics frameworks, the differences between popular memory forensics frameworks and other tools, and the impacts of said differences on adopting the correct fuzzing techniques. Furthermore, due to the fact that memory forensics frameworks utilize relatively large memory images, fuzz testing these frameworks requires optimizations both in fuzzing and in parallelizing the tester. Attendees will learn additional distribution techniques and how significantly they affect the efficiency of fuzz testing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing an open-source fuzzing tool that can test memory forensics frameworks and uncover issues that may potentially lead to inaccurate forensic results by causing the memory forensics tools to crash or behave unexpectedly. Consequently, the frameworks will become more robust by solving these issues, and the quality and efficiency of memory forensics as a whole will be improved.

Memory forensics is on a path to automation and its tools need to be more robust. Volatility is a popular framework used regularly in investigations, and in order to properly implement automated analysis, the ability to discover critical vulnerabilities that might arise must be included. Fuzz testing is a technique in which the program is intentionally fed flawed data to discover whether it enters an unexpected state. Due to the nature of these images, their mutations are tailored to the frameworks. The memory forensics frameworks are written in Python and are provided with one image file. The preferred fuzzing technique in terms of performance and efficiency is intercepting system calls using LD\_PRELOAD. This also provides the fuzzer the portability and flexibility to test other programs.

Fuzzing involves thousands of mutations on a single file and testing various plugins of the memory forensics framework on those mutations. This requires copious amounts of computing time and energy. Because of this, the tests were efficiently distributed using HPX. HPX is a general purpose C++ runtime system for parallel and distributed applications of any scale, and it has the capability of being adjusted to parallelize computation-heavy applications such as fuzzer.<sup>1</sup> HPX futures, for example, provide the possibility to set up work to be performed, fire it off, and wait for it to be finished. HPX takes care of creating the threads, moving the work across node boundaries, and making sure the calling thread suspends when it wants the value from a future that is still executing, provided that for any function wrapped with a future, any parameters of those functions are serializable.

The results of this research are presented in two aspects: the performance of the fuzzer and how efficiently it can test a memory forensics framework, and its findings during the tests. These findings will demonstrate how effective the fuzzer can be in memory forensics and in helping improve the frameworks, hence, making forensics more reliable and efficient. This fuzzer can be useful for other researchers to test other memory forensics frameworks and to help eliminate defects from them.

#### Reference(s):

<sup>1</sup>. <http://stellar.cct.lsu.edu/projects/hpx/>.

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**Memory Forensics, High Performance Computing, Fuzzing**



C36 WITHDRAWN



### C37 Machine Learning to Detect and Localize Forensics-Relevant Features

*Sara Mousavi, Knoxville, TN 37909; Audris Mockus, PhD\*, University of Tennessee, Knoxville, Knoxville, TN 37996-2250; Dawnie W. Steadman, PhD, University of Tennessee, Knoxville, TN 37996; Angela M. Dautartas, PhD, University of Tennessee, Knoxville, TN 37996-1525*

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**Learning Overview:** After attending this presentation, attendees will understand opportunities afforded by machine learning for automatic detection and localization of forensically relevant features in images documenting human decomposition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing new approaches to automate the annotation of digital collections and by discussing how this can transform research on human decomposition.

Rich forensic content in large digital media collections needs to be extracted in order to advance forensic science; for example, to obtain frequencies of phenomena in the population at large, and, more generally, to make forensic work more effective. Since manual extraction by forensic experts is not feasible for large collections, scientists seek to employ machine learning to detect and localize forensic features in the partially annotated collection of images taken from bodies during their decomposition process at the Anthropology Research Facility (ARF) at the University of Tennessee, Knoxville.

The initial nomenclature and annotation of forensic features in this collection was performed by human decomposition experts and produced approximately 5,000 annotations on 1,000 images so far. These annotations were used to train Convolutional Neural Network (CNN) models and identify areas in the remaining one million unannotated images using these models.

CNN models have revolutionized the field of image analysis, but they require massive numbers of training examples to train accurate models for a specific domain. This study is not aware of any attempts to use them in the forensic domain, in particular in recognizing features of human decomposition.

The forensic community faces several challenges, including uncontrolled natural lighting conditions that vary with weather and seasons, angles and distances at which the photos were taken, different areas of the body that the photos capture, and the difficulty of identifying and localizing the features, even by an expert in human decomposition. Furthermore, there is the need to localize features within an image and, most importantly, thousands of examples of each feature needed to train an accurate CNN model are not available.

Transfer learning, due to the absence of models suitable for forensic features, does not solve the problem. Instead, a hierarchical workflow was constructed that first normalizes the data and equalizes the images to even the distribution of intensity levels. The scale of the images was calculated by using existing CNNs to recognize body parts (using OpenPose), and, finally, sections of the images that do not contain the body were excluded. The preprocessing allows improved training and detection by focusing on specific scale and excluding features that could not be there, for example, signs of scavenging on non-muscular areas of the body, such as skin (scavengers tend to target muscles more than other areas). Existing images are then augmented by moving/resizing the area with annotation and rotating and rescaling annotated images. Mask-RCNN is applied to this normalized and augmented dataset to recognize and localize forensic features.

In preliminary work, highly complex features were recognized, such as signs of scavenging, with 36% accuracy even before fully applying the aforementioned preprocessing techniques and 38% accuracy when normalizing the dataset. The results suggest that CNNs can be successfully applied in the forensic context, even with a limited number of training examples, and that forensic content can be extracted from massive media collections, thus providing novel tools for advancing forensic science. Further work is needed to increase the accuracy of the models and create models for more forensic features.

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#### Machine Learning, Decomposition, Taphonomy



### C38 An Investigation of Distinctiveness of Skin Texture for Forensic Applications

*Frodo Chan, PhD, Hong Kong, HONG KONG SAR; Xiaojie Li, PhD, Nanyang Technological University, Singapore, SINGAPORE; Wojciech Matkowski\*, School of Computer Science and Engineering, Singapore 639798, SINGAPORE; Adams Wai Kin Kong, PhD, Singapore 639798, SINGAPORE*

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**Learning Overview:** After attending this presentation, attendees will better understand the distinctiveness of skin texture for criminal and victim identification based on digital images.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a key aspect of personal identification, as it can provide a new way to support the identification of suspects and victims whose faces and tattoos are not in the images (e.g., child sexual abuse or terrorist images).

Skin texture suggested for personal identification a decade ago has been neglected by the biometric community for a long time because of a low distinctiveness and unobvious features, similar to other soft biometrics and different from other hard skin biometrics, such as fingerprints and palm prints. However, in some cases, such as identifying terrorists, rioters, or pedophiles in images, where faces are masked or blurred and other traits such as tattoos or skin marks are not visible, skin texture may remain the only possible choice. Blood vessels, which are considered a hard biometric trait, have been suggested to tackle these problems. Nevertheless, successful visualization of blood vessels hidden in color images and consequently identification performance highly depends on image quality. Visualized blood vessels can be partially evident or not evident in low-resolution images, whereas skin texture, such as the forearm or thigh skin, are still visible in low-resolution images and may be useful clues for forensic applications.

A skin texture identification algorithm, which includes a positive sample generation scheme, dynamic and directional grids, large feature set generation scheme, and a classification using partial least squares regression is proposed to explore the distinguishing trait of low-resolution skin texture. The proposed algorithm is fully automatic and does not require any manual or time-consuming human supervision. To evaluate the algorithm, the image databases were collected in a laboratory environment and the internet to simulate more realistic scenarios. The databases contain more than 6,300 inner forearm and thigh skin images, which have a large pose, viewpoint, and illumination variations.

In the experiments, the proposed algorithm and the state-of-the-art texture recognition methods were evaluated. Moreover, the traits of skin texture and blood vessels, extracted from color and infrared image, were compared. First, the experimental results revealed that the proposed algorithm achieves significantly higher performance than the state-of-the-art texture recognition methods. Second, the comparison between skin texture and blood vessel distinctiveness demonstrated a superior performance of skin texture, revealing its potential usage in forensic applications.

*This work is partially supported by the Ministry of Education, Singapore through Academic Research Fund Tier 2, MOE2016-T2-1-042(S).*

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**Skin Texture, Criminal and Victim Identification, Biometrics**



### C39 A Study of Wrist Identification for Forensic Applications

Wojciech Matkowski\*, *School of Computer Science and Engineering, Singapore 639798, SINGAPORE*; Frodo Chan, *PhD, Hong Kong, HONG KONG SAR*; Adams Wai Kin Kong, *PhD, Singapore 639798, SINGAPORE*

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**Learning Overview:** After attending this presentation, attendees will understand how wrist images may serve as a biometric trait for criminal and victim identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding a new aspect of personal identification and provide a new way to support the identification of suspects and victims, whose faces and tattoos are not in the images, but whose wrists are still visible (e.g., child sexual abuse, rioters, or terrorist images).

To avoid being identified in digital images, criminals may intentionally hide their faces or tattoos. Biometric traits such as veins, skin marks, height, skin color, weight, race, etc., are used by some of the existing methods to solve this problem. Although soft biometric traits, including gender, race, height, weight, or skin color, offer useful information, they are not distinctive enough. Using skin marks and veins requires good quality, high-resolution images, which in some cases may be unobtainable. Even though good quality images are provided, body parts may not have enough skin marks or clear veins, or they may not be visible enough (e.g., wearing long sleeves). On the other hand, an individual's wrist can still be visible in these images. Terrorists and rioters expose their wrists in gestures of triumph, greetings, salutes, or when holding weapons; whereas pedophiles may show their wrists when touching victims. Wrists were neglected by the biometric community though, and per this study's research, no wrist identification algorithm for forensic applications has been proposed.

To explore the potential of wrists for forensic applications, a wrist identification algorithm, which includes skin segmentation, key point localization, image to template alignment, large feature set extraction, classification and post-recognition score analysis, is proposed. The proposed algorithm and six state-of-the-art biometric methods designed for palm print, palm vein, and fingerprint matching are evaluated on NTU-Wrist-Image-Database-v1, which consists of 3,945 images from 731 different wrists, including 205 pairs of wrist images collected from the internet, taken under uneven illuminations with different poses and resolutions. The database also includes two different images of a masked rioter who exposed his wrist and was photographed by an Associated Press photographer during the Baltimore, MD, riots in 2015.

The experimental results demonstrate that the proposed algorithm outperforms the state-of-the-art palm print, palm vein, and fingerprint matching methods. Moreover, the proposed algorithm successfully matches the masked Baltimore rioter within top ten ranks. In particular, he is retrieved at the first and eighth rank, depending on the algorithm parameter. This example and other experimental results demonstrate that a wrist is a useful clue for forensic investigation.

*This work is partially supported by the Ministry of Education, Singapore through Academic Research Fund Tier 2, MOE2016-T2-1-042(S).*

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#### Wrist, Criminal and Victim Identification, Biometrics



### C40 Methods to Characterize 3D Scanners for Forensic Applications

Prem Rachakonda\*, Gaithersburg, MD; Bala Muralikrishnan, PhD\*, Gaithersburg, MD 20899

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**Learning Overview:** After attending this presentation, attendees will better understand: (1) the issues in using 3D scanners for forensic applications; and (2) the National Institute of Standards and Technology's (NIST) efforts to develop procedures to evaluate 3D scanners.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating attendees about 3D scanning errors, scanning performance, and methods to measure performance. Such methods will lead to a greater confidence in scanner measurements.

Forensic photography has been one of the most valuable tools for investigators over many decades. Photographs preserve the history of the scene and provide a way for investigators to analyze the scene of interest. However, it is difficult to obtain any objective measurements with high confidence from such photographs.

3D scanning instruments such as Light Detection and Ranging (LiDAR)/Terrestrial Laser Scanners (TLS), Structured Light Scanners (SLS), and photogrammetry instruments digitize the scene of interest, offering a way for investigators to revisit the scene. Such a capability is crucial for investigators when access to the scene of interest and time may be limited when performing a thorough investigation. It is also possible that the scene itself may become polluted with the passage of time. These scanners enable investigators to obtain the scene information quickly and measure many features, such as a trajectory of a bullet, track width of a vehicle, etc.

The new generation of 3D scanners are portable, easy to use, and capture millions of points in a matter of seconds. Apart from capturing 3D data, these scanners also capture the intensity and/or color of the scene and provide a way to reconstruct the scene in any desired orientation. 3D scanners do suffer from some issues that can cloud the accuracy of their measurements. When used in forensic applications, such confusion can affect the standard of admissibility of evidence in judicial hearings, such as *Daubert* or *Frye* proceedings. The inaccuracies in these 3D scanners may come from their construction, data analysis procedures, calibration artifacts, or environment. Such inaccuracies must be characterized before using these devices the field.

To standardize the evaluation of a class of TLSs, the Dimensional Metrology Group (DMG) at the NIST has worked with instrument manufacturers, end users, and experts to develop a new documentary standard (ASTM E3125-17). This standard was developed under the auspices of the American Society for Testing and Materials (ASTM) and was published in December 2017. The NIST was a major contributing member of this effort and possesses unique expertise for such activities due to prior experience with other 3D measuring instruments. A considerable amount of research was conducted to unravel the sources of errors in these instruments and a variety of procedures and software algorithms were developed to minimize the effects of these errors on the data obtained. Over 20 publications were generated out of this activity, detailing various issues that affect the scanner performance and ways to minimize those issues.

In this talk, the errors and accuracy of TLSs and other efforts to characterize the performance of TLS systems to ensure that they meet the needs of the forensics community will be discussed.

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#### Forensic 3D Scanning, Accuracies, Standards





### C41 A Ladder Logic Decompiler for Supervisory Control and Data Acquisition (SCADA) Network Forensics

*Irfan Ahmed, PhD\*, Virginia Commonwealth University, Richmond, VA 23284*

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**Learning Overview:** After attending this presentation, attendees will understand a decompilation process of a binary ladder logic program under three cyberattack scenarios to support the network forensic analysis of SCADA systems.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing a new decompiler for a ladder logic program, which is useful for SCADA network forensics, and allows a forensic investigator to scan the network traffic dump of a SCADA network, identify any transfer of a ladder logic, further retrieve the logic from the dump, and analyze it using the decompiler.

An attacker can transfer a malicious program over the network to compromise a given target Programmable Logic Controller (PLC). PLC is an essential and critical component for the automation of industrial processes, such as gas pipeline, chemical and nuclear plants, and power generation and distribution. It has a control logic that defines how a PLC controls the actuators based on input devices, such as sensors. For instance, a ladder logic program can communicate to a PLC to turn off a water pump when water reaches a given level in a tank. Ladder logic is a popular programming language for PLCs. It consists of graphical symbols, which are placed together in AND/OR logic sequence to write a control logic. During an investigation, if forensic investigators find a ladder logic program in a network traffic dump, they can further extract the program from the dump and then decompile it for further forensic analysis.

Interestingly, when a program is compiled, RSLogix does not create its low-level representation of a ladder logic program on disk (such as an executable) that is used to run on a PLC. However, when RSLogix downloads a program to a PLC, it transfers the low-level representation that can be captured and extracted from the network traffic, which can be decompiled to understand the logic actually being transferred to a PLC. This approach is useful for SCADA network forensics. In a case of an incident, a forensic investigator can scan the network traffic dump of a SCADA network, identify any transfer of a ladder logic, further retrieve the logic from the dump, and analyze it using a decompiler.

In particular, the decompiler first identifies the start and end of each rung in a program and, further, parses each rung to identify a sequence of instructions and their AND/OR relationship. Differential analysis is used to map low-level representation with higher level, such as unique sequence of bytes that represent each graphical symbol. The decompiler is equipped with a comprehensive database of the mapping. This presentation also discusses a number of challenges in developing the decompiler, including developing signatures to identify the start and end of a rung, format of each instruction, which varies in size and is analogous to opcode and operand of Intel Assembly. Opcode in a ladder logic instruction has a specific byte-sequence; however, the operand may have a different representation with respect to the opcode.

A prototype implementation (tool) of the decompiler will be discussed under three cyberattack scenarios in which an attacker either hides a malicious control logic from RSLogix or compromises the RSLogix's ability to retrieve the logic from a compromised PLC remotely. The tool can analyze the network traffic and reveal any control logic (at source code level) present within the traffic.

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#### Ladder Logic, SCADA Forensics, Network Forensics

## D1 The Application of Optical Coherence Tomography (OCT) in the Detection of Latent Fingerprints

Ning Zhang\*, Beijing, CHINA; Chenming Wang, Tsinghua University, Beijing 100084, CHINA; Zhigang Li, MD, Institute of Forensic Science, China, Beijing 100038, CHINA; Zhihui Li, PhD, Beijing, CHINA; Wei Huang, Institute of Forensic Science, Beijing 100038, CHINA

**Learning Overview:** The goals of this presentation are to show how to: (1) detect latent fingerprints hidden beneath adhesive tape, and (2) separate overlapped fingerprints by a novel forensic imaging technique, OCT.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a novel forensic imaging technique, OCT, into the detection of latent fingerprints, which enables obtaining cross-sectional structure while being non-invasive, *in situ*, high resolution, and high speed. OCT may become a reliable guide and complementary method in forensic investigation.

Fingerprints are usually covered by a certain non-transparent material at a crime scene, which leads to difficulties in detecting and matching the fingerprint. For example, the fingerprint can be deposited unintentionally on the sticky side of adhesive tape by the suspect in various criminal cases, including rape, murder, kidnapping, and explosives.<sup>1</sup> However, the adhesive tapes found at crime scenes are often stuck together or attached to a certain substrate. Consequently, the latent fingerprints are sandwiched between two strips of adhesive tapes or between the tape and the substrate, making the prints difficult to detect and visualize. Traditional treatments, such as mechanical separation, steam heat, chemicals, liquid nitrogen, and freezing with refrigeration, would more or less affect the physical condition of tapes, contaminate the biological evidence, and eventually may adversely affect the accuracy of identification.<sup>2-4</sup> In addition, latent overlapped fingerprints are frequently encountered in latent fingerprints lifted from crime scenes, which are difficult to separate and match by fingerprint matchers.<sup>5</sup> A few methods have been proposed to separate overlapped fingerprints, but these methods are not accurate and reliable enough.<sup>6</sup> It is desired to develop a non-invasive and high-resolution subsurface imaging method to reduce the labor of fingerprint examiners.

OCT is an optical imaging technique that is new in forensics and performs high-resolution, cross-sectional tomographic imaging of the internal microstructure in materials and biologic systems by measuring backscattered or back-reflected light.<sup>7-8</sup> In the first part of this presentation, a custom-built OCT system was employed to detect and visualize the latent fingerprints that were deposited on the boundary of adhesive-substrate *in-situ*, without any pre-treatments, such as unraveling or chemical processing, preserving the integrity of tape evidence. Three most commonly used types of adhesive tapes (i.e., electrical tapes, box-sealing tapes, and Scotch® tapes, were chosen, and nine different samples were prepared for obtaining the internal fingerprints. The OCT system employed a broadband, super-luminescent diode as a light source centered at 832nm with the Full Width Half Maximum (FWHM) bandwidth of 60.4nm. The system was tested to achieve ~6µm resolution and the maximum imaging speed of 70 kHz A-line rate that equals to 43fps. For 3D image acquisition, single Axial scan (A-scan) acquisition was combined with 2D lateral scanning mechanism. A hand-held probe was designed to realize portable and flexible scanning across an area of the samples. The *en face* (transverse section) images of each sample at the subsurface layer of the latent fingerprints were presented. The results demonstrated that OCT can rapidly detect and recover precision images of latent fingerprints hidden beneath adhesive tapes while maintaining the original physical and chemical state of the sample.

In the second part of this presentation, the same OCT system was adopted to separate four overlapped fingerprints, which were deposited on the slide glasses oriented at 0°, 90°, 180°, and 270°. 3D OCT reconstructions were implemented and *en face* images of the fingerprints at each subsurface layer was extracted from the 3D OCT dataset. The individual fingerprints were successfully recovered in high resolution due to the tomographic ability of OCT. The results show that OCT provides a reliable guide and complementary method to separate overlapped fingerprints in a non-destructive and fast detection manner.

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### Fingerprint Detection, Optical Coherence Tomography, Forensic Imaging



### D2 Would Blunt Blades Cut Knife Crime?

Sarah V. Hainsworth, PhD\*, Aston University, Birmingham B4 7RT, UNITED KINGDOM; Patrick H. Geoghegan, PhD, Aston University, School of Life & Health Sciences, Birmingham B4 7ET, UNITED KINGDOM; Guy N. Rutty, MD, University of Leicester, Leicester LE2 7LX, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will understand how a knife tip profile affects the penetration ability of knives in stabbing attacks.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how knives can be manufactured or altered to modify the tip profile and to make them less dangerous as weapons.

Knife crime continues to be a significant issue in the United Kingdom and other countries where guns are not readily available. In 2009, Deputy Chief Constable Alf Hitchcock of the Metropolitan police, the National Lead for Knife Crime, was quoted as saying, “Knife manufacturers can reduce deaths on our streets by making blades with blunt tips.” This referred to research conducted by Hainsworth, Delaney, and Rutty that showed that sharpness of the tip of a knife was important in determining how much force was required for it to penetrate into the body.<sup>1</sup> More recently, retiring Luton Crown Court Judge Mudge proposed a nationwide program to file down the points of kitchen knives to solve the “country’s soaring knife crime epidemic.”<sup>2</sup> In his valedictory address, Judge Mudge called on the government to ban the sale of large, pointed kitchen knives over his concern that carrying a knife had become routine in some circles. The Judge noted that “the use of headline grabbing Rambo knives and samurai swords was relatively rare and it was the points of ordinary kitchen knives that was causing the soaring loss of life among youngsters.”<sup>2</sup> The Judge went on to “urge all those with any role in relation to knives—manufacturers, shops, the police, local authorities, the government—to consider preventing the sale of long pointed knives, except in rare, defined circumstances, and replacing such knives with rounded ends.”<sup>2</sup> He further suggested that “the police could organize a program whereby the owners of kitchen knives, which have been properly and lawfully bought for culinary purposes, could be taken somewhere to be modified, with the points being ground down into rounded ends.”<sup>2</sup>

Therefore, the goal of the research in this presentation is to determine the ideal tip on a knife to make kitchen knives less effective as stabbing implements. Ordinary kitchen knives have had their tips modified; this study has tested their ability to penetrate the skin when used for stabbing. The research took ten ordinary kitchen knives available from commercial manufacturers and changed the tip profiles by grinding the ends. A series of tests have been conducted with the knives using quasi-static penetration testing to test the force required for penetration with the varying knife profiles and stabbing into a dynamometer to see whether penetration of pork skin was possible with the modified knife tip profiles and recording the force that was required.

In addition to testing the knives for penetration, this study assessed the utility of the knives as kitchen implements after removing the tip, with a series of user trials.

The results show that rounding the tips of knives is effective in reducing their ability to be used as effective stabbing weapons, while demonstrating that they are still effective for cutting in domestic kitchen usage.

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#### Stabbing, Knives, Sharpness

### D3 Understanding the Difference Between Expired and Impact Bloodstain Patterns

Patrick H. Geoghegan, PhD\*, Aston University, School of Life & Health Sciences, Birmingham B4 7ET, UNITED KINGDOM; Erik Van Zuijlen, BSc, University of Applied Sciences van Hall Larenstein, Leeuwarden 8901 BV, NETHERLANDS; Loek J. Van Steijn, BSc, Amsterdam, Noord Holland, NETHERLANDS; Michael Taylor, PhD, Christchurch, NEW ZEALAND; Mark C. Jermy, PhD, Department Mechanical Engineering, University of Canterbury, Christchurch 8140, NEW ZEALAND

**Learning Overview:** After attending this presentation, attendees will understand that certain factors can provide a better indication of whether a spatter pattern occurred from impact and gunshot or expired events. Attendees will also understand the challenges in differentiating impact and gunshot patterns from expired spatter patterns.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing new data that can be used to better understand the differences between expired and impact spatter patterns that can be used when performing crime scene investigations.

Correct classification of the mechanism causing a bloodstain pattern is of importance within Bloodstain Pattern Analysis (BPA). The characteristics of bloodstain patterns that have been created by expiratory mechanisms are not properly defined. Without mucus strands, oral bacteria, or vacuoles, it is difficult to distinguish these bloodstain patterns from other patterns that look very similar, such as patterns created as result of an impact event. This work aims to determine characteristics within bloodstain patterns created by expiratory mechanisms, which differ from characteristics in bloodstain patterns created as a result of an impact event.

A Blood-Mimicking Fluid (BMF) similar to that described by Stotesbury et al. comprised of liquid honey, pasteurized egg white, red dye, and de-ionized water was utilized in each mechanism investigated.<sup>1</sup> The expectorate mechanisms investigated were blowing, speaking (normal voice), speaking (raised voice), sneezing, involuntary coughing, voluntary coughing, and exhalation. Blunt force impact spatter patterns were created for comparison with the expired patterns by swinging a selection of typical blunt force impact weapons by hand into a pool of 2.5ml BMF. The weapons were a ballpeen hammer, baseball bat, claw hammer, crowbar, a piece of timber, a rubber mallet, and an iron tube. Gunshot patterns were generated by shooting a chicken breast wrapped in a BMF-soaked T-shirt with a .45 caliber projectile.

All BMF trajectories for each event investigated were recorded using a Photron FASTCAM SA5 high speed camera and a Tamron 25mm–75mm zoom lens recording at 10,000fps. Multiple 10,000-lumen LED lights provided the illumination. Target surfaces for the impact patterns were provided by sheets of card placed at different distances, dependent on the mechanism investigated. Impact patterns were photographed using a Canon® EOS 5D Mark III with a Nikon® 50mm lens. Analysis was performed using the software program ImageJ to determine the number of stains and the stain size. The velocities of the expiratory mechanisms were also measured using the same hotwire anemometry technique described by Geoghegan et al.<sup>2</sup>

Although it cannot be definitively stated that impact and gunshot patterns can be distinguished from expired spatter patterns, there are some indications that make a pattern more likely to be an impact, gunshot, or an expired spatter pattern. These indications were found in the shape of the pattern, the number of stains on the target surface, stain size distribution, downward-pointing directional-shaped stains, and linear-shaped stains. Expired spatter patterns that created less than 500 stains, typically low velocity mechanisms, and with an unrecognizable-shaped pattern were most likely to be confused with impact stains with less than 500 stains created using a ballpeen hammer.

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#### Expired, Impact, Blood Pattern Analysis



### D4 Buffy the Vampire Slayer: But How Much Force Did She Need?

*Sarah V. Hainsworth, PhD\**, Aston University, Birmingham B4 7RT, UNITED KINGDOM; *Patrick H. Geoghegan, PhD*, Aston University, School of Life & Health Sciences, Birmingham B4 7ET, UNITED KINGDOM

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**Learning Overview:** After attending this presentation, attendees will understand how stakes were used in history in deviant burials to prevent corpses from rising from the grave or to inflict postmortem punishment. Attendees will also understand what materials were used in staking and what forces are required for staking bodies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how stakings were performed and the factors affecting the choice of stakes and staking methodologies.

Staking of bodies was used in medieval times, particularly in southern Slavic regions, to prevent corpses rising from the grave. The process of decomposition of bodies was poorly understood and exhumed bodies were considered unnatural if they had not decayed or were apparently fatter than when alive. Thus, staking of bodies was ritually used to prevent vampire epidemics.

The practice of staking was also found at sites of “deviant burials” where the corpse was treated in a ritual way. The corpses typically belonged to criminals or blasphemers or those that didn’t fit in with society. The earliest evidence for staking was from Dolné Věstonice in Moravia (dating from 25,000 BC) where a thick wooden pole went through the thigh. Staking was also seen for an Iron Age bog body in Ireland dating from c362–175 BC, where the upper arms were pierced with a sharp implement and hazel rods were inserted. A coffin found in Metholoni, Greece, contained an adult male nailed to the coffin with metal spikes.

In the 1950s, a 6<sup>th</sup>-century skeleton was found near an ancient church site in Southwell, Nottinghamshire, United Kingdom. The remains had been ritually staked. Iron nails were used to pierce the shoulders, heart, and ankles, and the body was in unhallowed, swampy ground outside the churchyard. In the United Kingdom, suicides were buried with a stake through their heart until an Act of Parliament abolished the practice in 1823. Suicides were considered to be guilty of *felo de se*, crime against the self. The bodies were taken and buried at rural crossroads. Suicide remained a crime in the United Kingdom until 1961.

This work examines the stakes that have been found in a variety of archaeological digs, and from complementary museum exhibits, and considers how much force would have been required to drive the stakes into the corpse and pin the bodies to the ground. Stakes that were used in these types of rituals were typically either iron or wood; 2cm-diameter, 14cm-long iron nails have been retrieved from digs in Bulgaria. Wooden stakes were typically made from ash, hawthorn, or juniper, and were larger, with dimensions approximately 30cm long by 3.5cm diameter.

Several stakes of differing materials have been manufactured and drop testing has been used to measure the force required to drive the stakes through a body by testing with a foam-silicone, rubber skin-simulant system. More slender stakes with higher cone angles at the tip penetrate the skin-simulant system more readily, while larger stakes with shallower cone angles require more force to penetrate.

This work provides insights into ways in which these ritual burials would have occurred.

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#### Staking, Forces, Penetration Resistance



### D5 Assessing the Construction and Performance Potential of Improvised Hand Grenades

*John Nixon, MBA\*, ARC, Bippus, IN 46713*

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**Learning Overview:** The goal of this presentation is to educate the forensic and legal communities regarding the technical and performance characteristics of improvised hand grenades.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping them appreciate the differences between professionally designed and manufactured military hand grenades versus those of improvised design and construction.

Television and movie productions have tended to exaggerate the effects of hand grenades to enhance visual impact and increase the dramatic effect of their productions. This has resulted in increased demand for hand grenades among the criminal fraternity. The better-organized criminals, such as Mexican drug cartels, may have relatively easy access to genuine military hand grenades that have been misappropriated from government storage facilities by theft, intimidation, or bribery. The more mundane small street gang or individual criminal who has a perceived need for hand grenades must improvise. This often results in personal injury and/or criminal prosecution under the various state and federal destructive device laws.

Hand grenades differ from other explosive devices in a number of ways, the key differences being the ability to be thrown and to have a short time delay—typically four to five seconds. Genuine hand grenades may be divided into two broad categories—defensive and offensive. As with most weapons, hand grenades have evolved over the years, and modern designs are both more effective and more flexible than older designs.

Defensive hand grenades produce metal fragmentation that causes injury to enemy combatants and may cause collateral damage to surrounding objects. Older grenade designs have thick-walled bodies that are designed to produce large fragments when they explode—the typical “pineapple” design often shown in World War II movies. More modern designs utilize a thin-walled body to contain the explosive charge, surrounded by a layer of notched wire or steel balls to produce injury-causing projectiles. The principal is that if you are defending a facility that is being attacked, you will have no concern regarding any collateral damage that may be inflicted outside the boundaries of that facility.

Offensive hand grenades, sometimes called stun grenades, are simply a thin-walled container filled with high explosive. They are designed to explode inside a structure—they produce heat, flash, extreme noise, and overpressure. The principle is to disorient facility defenders long enough to enable attacking forces to overwhelm them. There is little damage to the facility being taken, so few repairs are required, and the attacking force can acquire a viable asset. Some designs of offensive grenade incorporate smoke-generating ingredients or noxious odor-generating chemicals to enhance the disorienting effects upon the defending force.

The criminal element of modern society are not all “dumb criminals,” and there are many innovative ways to produce improvised hand grenades. However, limited access to high explosives and detonators means that the improvised devices typically have a far lower performance than their professionally designed and manufactured namesakes. Information on how to “build your own” can be found on the internet; however, the instructions are often vague, assume a degree of technical knowledge and skill on the part of the reader/builder, and may be incorrect in their assertions. The net result of this is that the devices being assembled are often of more danger to the constructor than to society. Methods to quantify the potential dangerous effects of these devices are available to forensic examiners and researchers. Case studies will be used to illustrate the points discussed.

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#### **Hand Grenades, Improvised, Destructive Device**



### D6 Handgun and Ammunition Performance Influencers

*John Nixon, MBA\*, ARC, Bippus, IN 46713*

**Learning Overview:** The goal of this presentation is to educate the forensic and legal communities regarding the technical and performance characteristics of handguns and ammunition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining how ammunition and handgun design features influence terminal performance observed in real-world shooting scenarios.

Forensic practitioners are often asked to categorize property damage, or wounds to victims, by the caliber of the firearm that caused them. Commonly, in situations where two handguns of different calibers are known to have been used, the forensic firearms practitioner may be asked to determine which handgun was responsible for each area of damage based upon the degree of damage sustained. This is not always an easy task because ammunition performance varies due to a number of factors related to design, materials used in manufacture, and the firearm in which it is discharged.

This is a complex subject, and it can be very confusing for the non-expert to comprehend because apparently “obvious” things are not really obvious at all. Examples would be caliber designations, where .38 Special and .44 Magnum are not actually 0.38" and 0.44" in diameter (often assumed to be so), or barrel lengths, where a 3" revolver barrel and a 3" semiautomatic barrel are actually substantially different in length.

The situation becomes more complicated when laypeople reason, correctly, that 45 is bigger than 40 and then assume, incorrectly, that 45 must be more potent than 40. Compounding the problem yet further, there can be significant performance differences between individual caliber loadings. The performance envelope of a “lesser” caliber may actually overlap with the next caliber in the performance ladder—a “light” 40 versus a “hot” 45, for example, and this can result in target damage overlap between calibers. This presentation will present performance in terms of calculations, charts, and practical examples.

Finally, once the internal and external ballistic performance characteristics have been evaluated and understood, the problems of projectile weight, materials, and construction must be considered. Hard-cast lead bullets that perform well on hard targets and bears, for example, are likely to pass right through softer targets, causing relatively little damage. Conversely, a soft lead hollow nose bullet that expands and causes significant wounding in soft targets will most likely be defeated by slightly harder targets.

It is not unusual for incident scene investigators to simply look up generalized performance numbers in ammunition manufacturers catalogs and use those figures as a basis of comparison. It should be noted that manufacturers test the ammunition in one specific barrel length, and that performance can vary dramatically with changing barrel length. Additionally, while United States manufactures almost always test revolver ammunition in a vented 4" test barrel, and semiautomatic ammunition in an unvented 4" barrel, the same cannot be assumed for offshore ammunition manufacturers, so great care needs to be taken when researching published performance data.

The results of research demonstrating differences exhibited between platform (revolver versus semiautomatic), barrel length, “hot” versus “light” loadings, and bullet designs will be presented. It is concluded that while carefully researched ammunition manufacturers’ published ammunition performance data may be used as a general guide to terminal performance, if accuracy is important, then there is no substitute for actual testing.

#### **Handgun, Ammunition, Terminal Performance**



### D7 Environmental Forensic Microscopy of Particles

*James Millette, PhD\*, Millette Technical Consultants, Lilburn, GA 30047*

**Learning Overview:** After attending this presentation, attendees will understand how the use of environmental forensic microscopy of particles can be used to identify particles in various dusts and help determine their provenance.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how environmental forensic microscopy can be used to determine the identity of the particles found in various dusts and comparing the analytical results with information obtained from a number of sources.

Accumulated information from more than 40 years of analyses of dust particles from residential homes, office buildings, and outdoor areas provides the ability to draw useful comparisons between what may be called “normal” dusts and dusts that occur in specialty facilities, such as computer centers, or those dusts that resulted from man-made and natural catastrophic events, such as the 9/11 World Trade Center (WTC) disaster and wildfires.

Analyses were performed on 72 samples of dust collected from homes in seven geographically diverse metropolitan areas in the United States. The analysts used a stereomicroscope, Polarized Light Microscopy (PLM), Scanning Electron Microscopy (SEM) that included Energy Dispersive X-ray Spectroscopy (EDS) elemental analysis, Transmission Electron Microscopy (TEM) that included EDS and Selected Area Electron Diffraction (SAED), and Fourier Transform Infrared (FTIR) microscopy as necessary. Over 90% of the samples contained the following common components: skin cells, soil minerals, plant fragments, hair, cotton fibers, and starch granules. In addition to the 72-sample study, hundreds of other samples of residential and office dusts have been examined from the United States. Components often found in these samples included those found in the 72-sample study and also included: wool fibers, pollen, fungal material, soot, synthetic fibers, glass fibers, paper fragments, ink/photocopy particles, construction debris, insect parts, rust/metal flakes, and aerosol particles (such as hair spray). The types of particles in residential and office dusts were also influenced by the proximity to certain industrial processes (such as spherical glassy particles of fly ash from coal-fired power plants and metallic spheres from some incineration facilities).

A sample of dust collected from a computer facility showed many components similar to residential and office dust but also contained zinc whiskers. Zinc whiskers form tiny conductive filaments in the presence of electromagnetic fields on steel surfaces that have been plated with zinc (galvanized) for corrosion protection.

The general composition of WTC dust was found to be primarily mineral wool (glass), gypsum, cement and calcium particles, cellulose, soot and char, silica, <1%–2% asbestos, less than 1% paint (some of it lead paint), metal, vermiculite flakes, and glass shards. Microscopic spherical iron particles generated from high-temperature, torch-cutting efforts that were performed during the rescue efforts were also found.

As expected, the dusts from homes affected by the particles produced by a wildfire contained higher levels of burnt materials, both black carbon soot and char. In some instances, electron microscopy was able to distinguish between the aciniform (appearance of a bunch of grapes) black carbon soot of the fire and aciniform-engineered carbon black.

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#### Microscope, Dust, Particulate





### D8 An Overview of Computational Linguistic Techniques for Forensic Purposes

Carole E. Chaski, PhD\*, ALIAS Technology, LLC, Georgetown, DE 19947

**Learning Overview:** After attending this presentation, attendees will be familiar with techniques used in computational linguistics projects, such as Google®, and how these techniques can be useful for forensic purposes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing the forensic uses of computational linguistics.

By its very nature, forensic science borrows from non-forensic science; the strongest forensic sciences, such as DNA identification and toxicology, were all respectable sciences before they were discovered to have profound use within investigations and thus became forensic DNA analysis and forensic toxicology (i.e., forensic sciences). This study presents computational linguistics as another non-forensic science that has forensic applications. Computational linguistics for forensic issues is relatively novel and is not represented in most crime laboratories, but it has been steadily growing since it was first introduced as a research paradigm in 1994, with results first reported in 1997.<sup>1</sup> Further, testimony using computational linguistics has been admitted under both *Daubert* and *Frye* standards.

Computational linguistics is the branch of linguistics that uses computer programs and statistical analysis to test theories of language structure and use. Linguistic theories create grammars of a language; computational linguistics uses this grammar to create software (called a parser) that analyzes the language, thus testing how well the grammar actually works on real data. Computational linguistics software has two functions: Type 1—a computer performs language-based tasks for a human; or Type 2—a computer helps humans use language and language-based analysis to perform other tasks. In tasks of type 1, the input is language and the output is still language. In tasks of type 2, the input is language and the output is something about the language, outside of language itself. The first tasks are meta-linguistic, (language about language) while the second tasks are extra-linguistic (language for something else).

**Using Language-Based Software to Obtain Linguistic Information:** The most famous example of type 1, metalinguistic tasks, is the search engine Google®. The search engine is run by a computer program that enables the machine to receive language as keywords, find and retrieve information, and output language in the form of a document set matching the keywords. In addition to search and information retrieval, other computational linguistics techniques include similarity detection (ranking most closely similar documents in a set), automatic summarization of documents, text classification, identification of the language(s) in a document, and machine translation.<sup>2-7</sup> For jurisprudence, both search and summarization can be useful in e-discovery, while similarity detection can be used for contract and motion reviews. For forensic digital examination, similarity detection can be used to find similar strings in assembly language, software languages in malware, and natural languages, while language identification can be useful in the examination of variable names in malware.<sup>8</sup> For criminalistics, text classification can be very useful in determining if a document is really the type of text that it seems to be (such as threats and suicide notes).<sup>9</sup>

**Using language-based software to get extra-linguistic information:** The type of computational linguistics in type 2 takes in language and outputs some fact or assessment related to the language behavior but outside language. Language analysis by machine can be used to automate personality assessment and to evaluate language for detection of mental disorders, such as delusional thinking, schizophrenia, and depression.<sup>10,11</sup> For forensic psychology, this type of software has an immediate use of presenting a quantitative “no skin in the game” evaluation with known error rates. Computational linguistics can automate credibility assessment or indications of deception in language; this possibility has immediate uses in forensic interviewing and interrogation.<sup>12</sup> Computational linguistics can be used to determine who authored a document.<sup>13-15</sup> For forensic document examination, determining the authorship can be useful in advancing a theory of case; automated tools also provide a known error rate. Computational linguistics can be used to determine the social demographics reflected in the language of a document, even if the author is unknown.<sup>16,17</sup> For criminalistics, narrowing the pool of potential suspects through social demographics can be a useful asset to an investigation. Further, automatic identification of terroristic messages helps criminal investigators monitor social media.<sup>18,19</sup>

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### Computational Linguistics, Forensic Science, Extra-Linguistic Investigation



### D9 An Investigation of Large-Scale Safety Accidents Using a 3D Scanner and a 3D Modeling Method

*Kwangsoo Choi, MA\*, Wonju-si, Gangwon-do 26460, SOUTH KOREA*

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**Learning Overview:** After attending this presentation, attendees will understand the feasibility of using a 3D scanner and a 3D modeling method as a tool for the analysis of building collapses and machinery collapses.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing an effective tool for the analysis of building collapses and machinery collapses.

At present, the space and size of buildings, mechanical structures, and public facilities are becoming increasingly enormous in line with global industrialization and urbanization trends. As a result, safety accidents have occurred in larger forms than before. In the case of a large-scale safety accident, the damage and loss can be huge, and the possibility of loss of life is significant. Furthermore, the quality of life is adversely affected by increasing the public's anxiety. For these reasons, there is a strong demand for clearly identifying the cause of large-scale accidents more quickly.

In the case of large-scale safety accidents, many investigators and significant time are required when investigating the accident site. Furthermore, there are risks of additional accidents, requirements to restore the building rapidly, and difficulties in approaching the investigation site because of the possibility of additional collapse. It is thus challenging to investigate large-scale safety accidents.

Even after a field investigation, various studies should be conducted in order to prevent the same type of accident. Also, additional investigation is necessary in some cases. However, it is generally difficult to preserve the accident site for a long period of time.

A 3D laser scanner is a good tool when capturing 3D geometry of civil infrastructure, creating an as-built representation of a large industrial complex, reconstructing a crime scene, or generating 3D data for integration into building information modeling. The 3D model consists of a point cloud of geometric samples on the surface of the subject. These points can then be used to extrapolate the shape of the subject. If color information is collected at each point, then the colors on the surface of the subject can also be determined.

The use of a 3D scanner and a 3D modeling method can solve problems such as personnel shortage, the difficulty of approaching the accident site, challenges related to additional investigation and research, etc. Location information has been acquired by high speed, wideband scanning using a 3D scanner at the accident site and reconstructing the collapsed building using a 3D modeling method. The 3D modeling method can reenact the sequence of the accident.

This presentation will show how to apply the 3D scanner and 3D modeling method in real cases to reenact the accident and reconstruct a collapsed building.

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#### **3D Scanner, 3D Modeling Method, Reconstructed Collapsed Building**



**D10 WITHDRAWN**



### D11 *In Vivo* Tribometry: An Analysis of Muscle Activity Using Electromyography (EMG) During a Passive Leg Drop

Marcus P. Besser, PhD\*, Pennsylvania State University Abington College, Abington, PA 19001-3900

**Learning Overview:** The goal of this presentation is to give the attendee an appreciation of the importance of considering muscle activity levels when using *in vivo* tribometric measures to quantify slip resistance.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how current tribometric methods are insufficient for quantifying barefoot friction, as surrogate barefoot test feet lack biofidelity. If *in vivo* methods are used, an understanding of the test subject's muscle activity and its effect on tribometric measurements is essential.

**Background:** Floor slip resistance is routinely evaluated using walkway tribometers. These devices use samples of the floor surface and outsole materials to measure utilized friction, either in a laboratory setting or *in situ*. When artificial outsole materials are being evaluated, it is feasible to obtain multiple samples of the material for testing. But difficulties arise when evaluating barefoot slip resistance. At present, there is no recognized surrogate for barefoot human skin. In order to develop such a surrogate, we need to evaluate a diverse population of human subjects *in vivo* to accurately determine the inter-subject variability in skin-slip resistance.

A StepMeter has been designed and constructed to allow *in vivo* testing of barefoot subjects in a controlled manner.<sup>1</sup> In this device, a seated subject has their leg and foot passively raised 2cm, then dropped onto the floor or a test surface. The test surface is inclined, and the test is repeated until the foot slips on contact, to determine the slip resistance of that foot-surface system. Before using this device to evaluate the larger population, this study was conducted to ensure that the seated subject did not involuntarily tense their leg, which may affect the determination of slip resistance. EMG was used to determine muscle activity of four muscles (tibialis anterior, gastrocnemius, rectus femoris, and biceps femoris) as a percentage of the subject's maximum voluntary contraction.

**Methods:** This study was approved by the Institutional Review Board (IRB) at Penn State University. Subjects were recruited from the student population at Penn State University Abington campus. Adult subjects were excluded if they had any right leg or foot pathology that would preclude participation. Informed consent was obtained, subjects donned shorts, and EMG electrodes were placed on the four muscles, using the locations described in a reference on surface EMG.<sup>2</sup> Maximum Voluntary Contractions (MVCs) were elicited from the seated subject by a researcher manually applying resistance as the subject was instructed to extend or flex "as hard as they can."

After MVC data were collected, subjects were seated in the StepMeter. Their leg was constrained with a modified knee brace to support the leg and prevent internal or external rotation of the calf. Ten "drop trials" were collected; the subject's foot was raised so that their heel was 2cm above the floor, then was dropped. Five second trials of EMG data were collected; the drop was initiated by the researcher at an arbitrary point during that five seconds and was varied from trial to trial to prevent subject anticipation of the drop.

**Results:** Data were collected on 27 subjects; one subject's data were not included in the analysis due to instrumentation problems.

EMG data (MVCs and drop test) were high-pass filtered (6Hz), rectified, and low-pass filtered (6Hz) to obtain a linear envelop. Each subject's data were normalized to 100% MVC. Means and standard deviations were calculated within subjects to evaluate intra-subject variability. Means and standard deviations were calculated across the subject population to evaluate muscle activity during passive leg drop. Intra- and inter-subject variability were evaluated. The posterior muscles showed much greater activity than did the anterior muscles, but this result was not consistent across all subjects. From the raw data, one can see significant background EMG activity on the posterior muscles on many subjects; it is thought that this may be an artifact of the brace used to control the lower leg.

**Conclusions:** For anterior muscles, the level of muscle activity is low enough to provide confidence that this activity will not affect evaluation of slip resistance for barefoot walking. While posterior muscles showed higher levels of activity, it is suspected that this is artifact, due to interference from the brace used to control leg movement, and muscle activity will not affect slip-resistance evaluation.

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#### Barefoot Slip Resistance, *In Vivo* Tribometry, Muscle Activity



**D12 WITHDRAWN**

### D13 Improvement of the Human Body Damage Evaluation Method by Computer Simulation: Verification of Infant Bone Properties Compared With Actual Experiments

Tomotaka Matsubara\*, University of Yamanashi, Kofu, Yamanashi 400-8511, JAPAN; Yasumi Ito, PhD, University of Yamanashi, Kofu 400-8511, JAPAN; Tatsuya Fukuoka, University of Yamanashi, Kofu, Yamanashi 400-8511, JAPAN; Ryuichi Yamada, University of Yamanashi, Kofu, Yamanashi 400-8511, JAPAN; Yoshiyuki Kagiyama, PhD, University of Yamanashi, Kofu-shi 400-0001, JAPAN; Tetsuya Nemoto, PhD, Obu 474-8522, JAPAN

**Learning Overview:** After attending this presentation, attendees will understand the advanced fracture risk evaluation method using computer simulation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an advanced fracture risk evaluation method using computer simulation.

For injuries to young children, quantitative evaluation of external forces is required to find the possibility of abuse and prove its criminality. Previous studies revealed that soft tissues, such as skin and muscle, have a large influence on human body damage by impact force, and soft tissue was taken into consideration in impact force measurements. Also, it is difficult to measure the mechanical properties of soft tissue and quantify the relationship between its thickness and buffering performance. However, the presence or absence of soft tissue is more important than its characteristics in assessing the fracture risk of impact force by hitting. The finite element method is a method of visually expressing the behavior by calculation, and computer simulation of pig tail fracture load by Finite Element Analysis (FEA) analysis revealed that, in Young's modulus of soft tissue, 0.8, 1.4 kgf/mm<sup>2</sup>, the fracture load was respectively 693, 710 N (2% difference). In thickness of soft tissue: 0.5, 1.5mm, the fracture load was respectively 690,705 N (2% difference). Fracture load in the absence of soft tissue was 585 N (18% difference, compared to 0.5mm). Therefore, the presence or absence influences the fracture load more than the soft tissue property. On the other hand, bone density and fracture load are closely related, and many studies reveal the importance of bone density such as Okuizumi et al: (Fracture Strength) [N]=501+2908 (BMD: Bone Mineral Density) [g/cm<sup>2</sup>].<sup>1</sup> In addition, a detailed risk evaluation method of fracture from an individual bone shape and bone density has been developed and is being used in the medical field in Japan. However, in the risk evaluation of bone fractures caused by blows, it is currently difficult to make detailed evaluations in consideration of an individual's physical characteristics.

The problems in evaluating advanced fracture risk using computer simulation are as follows: (1) it is not possible to quantitatively evaluate the influence of soft tissue; (2) the verification by actual bone is limited to the parts (femur, vertebral column, teeth), and the simulation accuracy of other parts is not sufficiently verified; and (3) it has not been verified whether sufficient simulation accuracy is also obtained for bones having different material properties, such as infant bone.

As mentioned above, point 1 has already been verified, and it is necessary to conduct research on points 2 and 3. Therefore, in this study, fracture experiments on young pig tails and numerical analysis by computer simulation were performed to investigate the simulation accuracy for infant bones with different material properties. After the fracture load was investigated by the static compression test using the material testing machine, numerical analysis was performed by the computer simulation using the FEA under the same conditions as the fracture experiment.

As a result, the average fracture load was 143 N at the jig tip angle 60° to 90° in actual experiments, and the fracture load was 151 N in the FEA. Therefore, in fracture risk evaluation using FEA, sufficient simulation accuracy could be confirmed, even with infant bones. This study also conducted a verification aimed at further improving the fracture risk evaluation method using FEA. From these results, the accuracy of the fracture risk evaluation method using computer simulation improved. As a future task, it is conceivable to establish a detailed model construction of an infant skull and establish an evaluation method of infant fracture risk that can be used for appraisal.

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**Fracture Risk Evaluation, FEM Analysis, Infant Bone**

### **D14 The Influence That Personal Attributes of Elderly Drivers Contribute to Driving Ability and Its Secular Change: An Analysis of Physical and Mental Functions, Living Conditions, and the Effects of Diseases and Medication**

*Yasumi Ito, PhD, University of Yamanashi, Kofu 400-8511, JAPAN; Tomoya Sakuragawa\*, University of Yamanashi, Kofu, Yamanashi 400-8511, JAPAN; Kei Sato, Shigakkan Univeristy, Obu, Aichi, JAPAN; Shota Sakaida, Toyoda Gosei Company Limited, Kiyosu, Aichi 452-8564, JAPAN; Yoshitomo Orino, Central Nippon Expressway Co, Ltd Hachioji Branch, Hachioji-shi, Tokyo 192-8648, JAPAN; Ryuichi Yamada, University of Yamanashi, Kofu, Yamanashi 400-8511, JAPAN; Ryota Okuwaki, University of Yamanashi, Kofu, Yamanashi 400-8511, JAPAN; Tsukasa Kouno, University of Yamanashi, Kofu, Yamanashi 400-8511, JAPAN; Natsumi Suzuki, University of Yamanashi, Kofu, Yamanashi 400-8511, JAPAN; Yoshiyuki Kagiya, PhD, University of Yamanashi, Kofu-shi 400-0001, JAPAN*

**Learning Overview:** After attending this presentation, attendees will understand there are items that are not present in accident statistics and accident databases, such as health condition, medication state, life attitude, etc., in the micro data, such as a written expert opinion of the accident case being caused by health conditions, etc.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that the health conditions of the elderly, the number of years of education, the history of falls, and living conditions all affect driving ability.

Today, Japan has a high proportion (26.7%) of people over 65 years of age in the total population and faces a super-aging society. Accordingly, accidents caused by elderly drivers have increased and become a social problem. The Cabinet Office predicts that the elderly population will continue to increase in the future. On the other hand, movement by car is an important means to support the autonomous living of the elderly, and those who do not drive or who quit driving are more likely to be at risk of developing dementia than those who are still driving. Since it has been reported that this doubles the dementia risk, it is undesirable to easily take driving away from the elderly.

Therefore, since 2008, in a senior driver support project of Fujikawaguchiko-machi, the relationship between the physical function, brain function, lifestyle, etc. of the elderly and driving ability and its secular change have been investigated. Regarding the relationship between personal attributes and driving ability in elderly people, a significant positive correlation was found between the number of years of education and driving ability. Since the number of years of schooling is thought to affect occupation and life thereafter, it suggests that the living conditions of the elderly affect driving ability. From the influence of secular change, it became clear that most elderly people improved or maintained their driving ability. However, as a result of analyzing personal attributes of elderly people whose driving ability has declined, it turned out there were accident records and fall experiences that did not occur in the past. In addition, due to the effects of accidents and falls, opportunities for regular exercise were also decreasing. From this, it became clear that the health condition of the elderly, the number of years of education, the history of falls, and the living conditions affect driving ability. If it is possible to investigate health and living conditions from a detailed analysis of actual traffic accidents, it is considered that there is a possibility that the correlation with the accident becomes clear.

Currently, there are several detailed traffic accident databases, including the National Automotive Sampling System (NASS/CDS). However, they do not contain data on health and living conditions.

Therefore, it became apparent there are items that are not present in the accident statistics and databases, such as health condition, medication, life attitude, etc., or in the micro data, such as written expert opinions of accident cases caused by health problems, etc. This study proposes adding this information to traffic accident databases, such as NASS-CDS. This would enable safe driving of elderly drivers for a long period of time by using the databases and including the attributes and characteristics that affect driving ability that are revealed from analyses of accidents caused by health conditions, etc. and would be useful for research to prevent accidents beforehand.

### **Health-Related Vehicle Collisions, Attributes of Elderly Drivers, Field Experiment**





### D15 A Computational Analysis of Traumatic Head Injuries Resulting From Falls and Impacts in Sports

*Michael D. Gilchrist, PhD, DEng\*, University College Dublin, Belfield, Dublin 4, IRELAND; Michael Thomas Connor, BAI, University College Dublin, Belfield, Dublin D4, IRELAND; Michio Clark, MSc, University College Dublin, Belfield, Dublin D4, IRELAND; Adrian McGoldrick, MB, BCh, BAO, The Turf Club, Kildare, IRELAND*

**Learning Overview:** The goal of this presentation is to explain the development and use of 3D computational simulation tools, specifically multibody dynamics and finite element analysis, with reference to equestrian fall accidents that resulted in head injury. These tools can equally be used to reconstruct other sport impact events, collisions, falls, and both workplace and road traffic accidents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing simulation tools that are powerful, allowing forensic engineers to quantify the forces, stresses, and strains sustained by a person or safety equipment during an impact, and to compare the relative severity of alternative accident scenarios without the need for extensive experimental laboratory facilities.

This presentation demonstrates and explains the use of advanced 3D computational simulation tools, specifically rigid multibody dynamics and finite element analysis, in reconstructing various sporting accidents that led to head injuries. While the analysis of concussive head injuries sustained during equestrian racing are a particular focus of this presentation, these same techniques can be used to investigate different injuries from other sports and general falls, as well as workplace and road traffic accidents.

Equestrian sports are popular worldwide, although racing jockeys have higher incidence rates of concussion than people competing in football, soccer, cycling, skiing, or boxing. The objective of this research is to improve the design, quality, and safety of helmets that are currently being worn to protect against head injury. Equestrian helmets are currently designed to pass certification standards that involve a linear drop test against a rigid steel surface. Concussions in equestrian sports, however, typically occur after being thrown from a horse and impacting relatively compliant surfaces, such as turf or sand, at an oblique angle. The mechanics of oblique impacts against compliant surfaces are more complex and require the use of advanced engineering simulation techniques. Understanding the event characteristics and mechanics that lead to concussion in equestrian sports has positive implications for tomorrow's equestrian helmets and associated certification standards.

In-depth reconstructions have been conducted on concussive and non-concussive cases for which high-quality video data was available. Cases in which multiple impacts occurred to a jockey's head (e.g., both a fall and a kick) were excluded from this research. Approximately 500 videos of accidents in which jockeys fell from their horses have been examined using the Kinovea video player. This, together with accident report forms, determined impact location, surface type, and initial velocity. Multibody dynamics simulations (Mathematical Dynamic Models (MADYMO)) were conducted based on body position, impact location, and velocity determined from video analysis. Impact velocity and trajectory angle of jockeys at the point of contact were obtained from these simulations. Corresponding accelerations can either be obtained by analyzing a succession of high-quality video frames or by using an instrumented head form under laboratory-controlled conditions. Linear and rotational acceleration time histories were subsequently used as input into a University College Dublin Brain Trauma Model to determine levels of maximum principal strain within the brain tissue.

The impacts were of long duration (>22ms) with low peak rotational acceleration, similar to collisions in ice hockey, but relatively high peak linear accelerations with magnitudes more comparable to American football and Australian rules football. The low rotational accelerations and long impact durations are due to the compliant turf surface. The relatively high linear accelerations were a consequence of the large amount of energy transferred to the jockeys' heads during impact. These results confirm that oblique impacts to a compliant surface can lead to concussion and that this head injury occurs at acceleration levels that are significantly lower and of longer duration than current safety thresholds set in equestrian standards. It is apparent that the current equestrian helmet standards and design do not necessarily account for the loading conditions associated with concussion.

#### Equestrian Accidents, Head Injury, Concussion

### D16 A Predictive Finite Element Human Head Model to Assist Forensic Scientists

Jean-Sébastien Raul\*, Strasbourg 67085, FRANCE; Caroline Deck, PhD, Strasbourg, AE 67000, FRANCE; Franck Meyer, PhD, Icube, Strasbourg 67000, FRANCE; Nicolas Bourdet, PhD, Icube, Strasbourg 67000, FRANCE; Blandine Schneddecker, MD, UML, Pointe à Pitre, GUADELOUPE; Remy Willinger, IMFS, Strasbourg, FRANCE

**Learning Overview:** The goal of this presentation is to present a predictive Finite Element Head Model (FEHM) and to illustrate how this tool could assist forensic scientists in the elucidation of traumatic head injury cases by using a brain injury criterion based on multiscale computation of axonal elongation of real-world head trauma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the possible use of the finite element method in head injury cases.

In forensic science, it is often difficult to corroborate the causation of a head injury as a result of an accident or an assault. Clinical examination of the injuries may provide knowledge about the mechanism of injury, but there is often uncertainty to discriminate the aftermath of injury due to a fall or blow and to estimate the plausibility of a given head trauma explanation. Head injury tolerance study is necessary to assess the brain injury risk for a given impact hypothesis during forensic investigation. Regardless of rapid advances in technology and knowledge in forensic science, it is still considerably difficult to describe the causation of injuries. The consequence of axonal injury leads to cognitive and permanent disabilities and fatalities in most Traumatic Brain Injuries (TBIs).<sup>1,2</sup> In both the severe and mild TBIs, Diffuse Axonal Injury (DAI) is the most common pathology.<sup>3</sup> DAI is characterized by dynamic tensile elongation of axonal fibers and consequential fiber rupture.<sup>4</sup> However, the structural signature of this microscopic-level injury is not easily captured by conventional medical imaging methods and the results are difficult in DAI diagnosis. Computational head models, on the other hand, can be deployed to study the DAI mechanism, especially for the definition of brain injury tolerance limits.<sup>5,6</sup>

The main objective of this study is to present a FEHM and explain how this tool could assist forensic scientists in the interpretation of traumatic head injury cases by using a brain injury criterion based on multiscale computation of axonal elongation of real-world head trauma.<sup>5</sup> First, the implementation of new medical imaging data coming from Diffusion Tensor Imaging (DTI) into the FEHM in order to mimic the main axon bundles was performed to improve the brain constitutive material law with more efficient heterogeneous anisotropic visco-hyper-elastic material law and enable it to compute axon elongation at the time of impact.<sup>7</sup> This model was then validated against all existing data available in the literature in terms of skull fracture as well as in terms of brain pressure and brain displacement.<sup>5</sup>

Further, well-documented head trauma cases were simulated by using this FEHM in order to derive head injury criteria.<sup>5,8</sup> Based on the statistical analysis of different intra-cerebral parameters, it was shown that axon strain was the most relevant candidate parameter to predict DAI. The proposed brain injury tolerance limit for a 50% risk of DAI has been established at 14.65% of axonal strain, resulting in good accordance with experimental studies based on cell culture.<sup>5,9,10</sup>

To demonstrate the ability of this advanced FEHM to localize neurological injuries, a well-documented case report is presented. The case report summarizes a 29-year-old construction worker of 95kg weight and a height of 1.82m. He was found unconscious on the floor by his colleagues, with his head on a wooden board. The victim fell from a scaffold at an indeterminate level. The first medical observations were reported as fainting, vomiting, left tympanic perforation, and ear hemorrhage. Scans were then performed and showed frontal bilateral contusions, left temporal contusions, blood in the left auditory canal and in the left mastoid cells, as well as a left petrous bone fractures with a left lambdoid suture disjunction. The victim left the hospital after five days but showed severe neuropsychological dysfunction and memory failure. Neurological evaluation was carried out after ten months of cerebral trauma and showed very important changes of cognitive functions (memory; lack of concentration, reasoning and planning; anxiety and depression). These functions are related to the cortical structures (associative neo-cortex) and sub-cortical structures and they require an efficient inter-hemispherical transfer via the corpus callosum. These disorders correspond with frontal, temporal, and corpus callosum injury. This case was then reconstructed numerically with the FEHM and a good correlation was found between the simulation and actual fracture pattern. Moreover, brain axonal strain calculated was higher than the 50% risk of DAI, which is in accordance with the case report of an unconscious victim. The location of the elements with the highest axonal strain values corresponds to the location of the corpus callosum and the frontal lobe and is coherent with the medical report.

HNBi N°3.8 Interreg V Rhin Supérieur project—FEDER.

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### Finite Element Head Model, Axonal Strain, Head Injury Prediction Tool



**D17 WITHDRAWN**

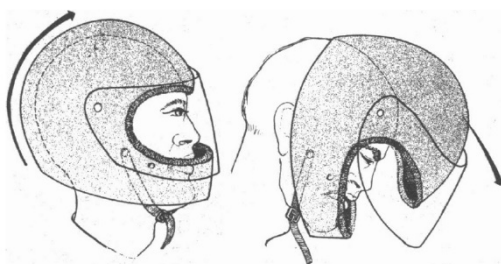
## D18 Mechanisms of Motorcycle Helmet Accident Retention Failures and Experimental Comparisons With a Safer Alternative Retention System Design Used in an Adult Football Helmet

Kenneth J. Saczalski, PhD, Newport Beach, CA 92661; Mark C. Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM 87015; Todd Saczalski, BSMET, TKS Consulting, Inc., Colorado Springs, CO, CO 80907

**Learning Overview:** This presentation presents methods for forensic analysis of motorcycle helmet detachment mechanisms during crashes and demonstrates an anthropomorphic head-impact test for comparison of defective retention systems with available alternative safer helmet designs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how and why helmets are displaced or ejected in collisions and alternative design retention systems that attenuate or eliminate loss of helmet performance and head protection.

Helmet protective capacity is diminished or non-existent if displaced or ejected. Helmet research on displacement/detachment during crashes was published by Richards and Hurt, et al.<sup>1,2</sup> The United Kingdom Department for Transport reported “helmet detachment ... in 10% to 14% of casualties.”<sup>3</sup> Restrained helmets can be significantly displaced or ejected off the head in motorcycle impacts, due to “loose fastening of the chin strap and possibly a loose-fitting helmet.”<sup>3</sup> Hurt noted “helmets properly fitted, retention system securely fastened, but the helmet is ejected.”<sup>2</sup> Richards diagrammed how properly fitted and securely fastened helmets can eject in frontal impacts where “the torso is slowed ... but forward inertia of the helmet, the geometric attachment of the chin strap system, plus head rotation, allows the helmet to roll off the head before impact with another vehicle, and/or other fixed objects.”<sup>1</sup>



Manner in which helmet is detached at high speed, showing (left) normal position and (right) helmet pivoting forward on strap during accident.

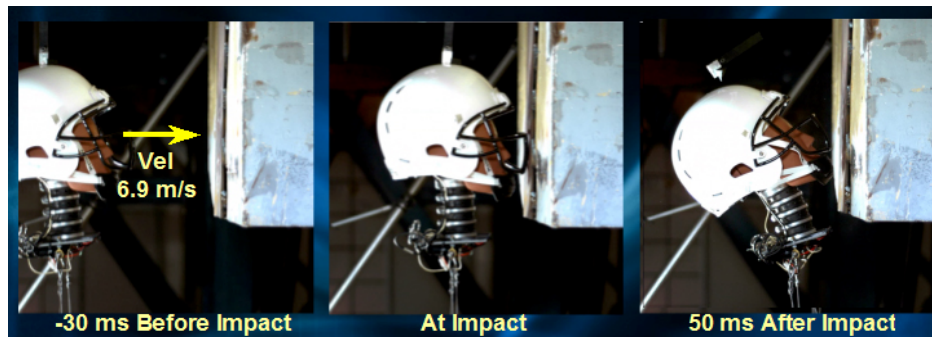
Another mechanism occurs if “head impact crushes front helmet padding, forward movement within the helmet causes a gap between the occiput and rear padding, loosening the helmet the same as if poorly fitted.<sup>1</sup> Additional severe head impacts, with the helmet misaligned or completely detached from the head, can occur. “Double D-ring” single chinstrap designs in use since the 1950s, (above,) cinch the helmet downward onto the top of the head, but the helmet is still free to rotate forward. “Full-face” helmets create additional fulcrum effects, increasing helmet rotation about the chin strap attachments. This was demonstrated by tests on open-face and full-face helmets securely attached to the head and neck of fifty percentile male Hybrid-III surrogates using a pendulum impact testing shown in a prior AAFS paper.<sup>1</sup> Video clips below demonstrate “roll-off” of a full-face helmet system 45msec after a 6.9m/s impact. (Note occipital area displaced from helmet.) This resulted in a 170J impact energy applied to the helmet frontal region. Resultant head acceleration was 296G and 15msec HIC was 732. The maximum pitch angular velocity was 31.5r/s.



A remedy for single chin strap defects was noted in identical testing of a football helmet retention design where a “two-strap” retention system was attached in a “Y”-shape manner, with one strap oriented from a “chin-cup” over the head apex (similar to the “Double D-ring” schematic) and another strap, oriented to the rear interior of the helmet so padding is pulled tightly beneath the occipital condyles, eliminating helmet rotation off the head.<sup>4</sup>

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Football helmets with face masks have a similar geometry to full-face motorcycle helmets. Impact to these face protection structures can have similar “fulcrum” effects on the helmet, causing displacement or ejection from the head. The figure below shows that this “Y” retention system design holds the football helmet securely to the head. Peak resultant acceleration was 219G and 15msec HIC was 518. Better-secured helmet mass provided reduced head pitch angular velocity of 26.1r/s, and a lower likelihood of rotationally induced head injury, versus higher 31.5r/s pitch rate of the motorcycle helmet.



Piozzi conducted tests using Hybrid III head/neck comparing motorcycle helmet single straps with “Y”-type straps from “shorty”-type motorcycle helmets and “Y”-type bicycle helmet straps. Significant improvements in helmet stability and retention were shown by “Y”-type straps.<sup>5</sup> Existing helmet Standards FMVSS 218, British Standard 6658, and ECE Standard 22.05 do not adequately evaluate these helmet retention deficiencies or head injury risks.

In summary, dynamic testing of helmet retention systems via this human responding head-neck and pendulum impact test method provides accurate head injury risk assessment of alternative safer retention systems. This enables proper forensic evaluation of helmet system retention performance and related causes of head injury, regardless of compliance with limited government safety standards.

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### Motorcycle Helmet Retention, Football Helmet Retention, Head Injury Severity



### D19 The Conspicuity of a Horizontal Rope Hazard in the Path of a Bicyclist: A Case Study

James B. Hyzer, PhD\*, Hyzer Research, Janesville, WI 53545-4077

**Learning Overview:** The goal of this presentation is to discuss how camouflage and depth perception influence the ability of a bicyclist to visually perceive and recognize a horizontal rope hazard suspended in his path.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by advancing the understanding of how conspicuity, camouflage, depth perception, and expectancy influence the probability that a bicyclist will not visually perceive a rope hazard in time to avoid being “clotheslined.”

The case study involves a 71-year-old male bicyclist, riding an upright bicycle into a white rope suspended horizontally at near neck height, between two lamp posts, across his intended path through a parking lot. An issue in the case is why the bicyclist did not see the rope that he hit.

It will be shown that even though a rope hazard may be in plain sight and visible to observers who know that it is there, to a rapidly approaching bicyclist that is not expecting it, a rope hazard must be sufficiently conspicuous relative to its background to be detected. Generally, the probability that a hazard will be detected increases with increased hazard conspicuity (how conspicuous it is). At the extremes, rope hazards that are highly conspicuous have the highest probability of being detected at the greatest possible distances, and hazards that are camouflaged may not be seen or recognized at all.

In addition to conspicuity, to be noticed, a rope needs to be perceived as being close enough to be considered a hazard. To the observer moving rapidly through a scene, primary critical cues to the perception of distance to a hazard come from motion parallax and binocular vision. It will be shown that a horizontal rope at near eye height to an approaching bicyclist will exhibit a visual illusion that make it difficult or impossible for the bicyclist to perceive as becoming hazardously close.

An earlier presentation addressed the conspicuity of wires ropes and cables under substantially ideal conditions, where the hazard is viewed against a uniform background and when the observer has sufficient time for observation.<sup>1</sup> For the subject case, factors that were only briefly discussed in the 1997 paper become primarily relevant for understanding why a bicyclist, exercising reasonable care with respect to lookout, failed to visually perceive a rope hazard. Specifically, it will be shown through case study that a bicyclist did not see an elevated, horizontal white rope hazard that he rode into for the following reasons: (1) the rope hazard was camouflaged against its background, making it difficult to visually recognize as a hazard in his path as he approached; (2) at near head height, the rope hazard did not exhibit significant parallax movement that would have offered a cue to changes in distance as the bicyclist rode toward it; (3) the distance to horizontal wires, ropes, and cables, without hazard warning flags or markers, is generally difficult or impossible to judge with horizontal binocular vision; and (4) the rope hazard was unexpected for the bicyclist, and as he rode across the parking lot toward his intended path, his focus was reasonably on the parking lot surface beyond it.

The rope in the case study at near the time of the accident was measured to be 58.5 inches above the pavement. The bicyclist’s neck height, while on his upright bicycle, was measured to be 61 to 62 inches. The United States Department of Transportation Federal Highway Administration (FHWA) reports an average eye height for a bicyclist as 60 inches. For comparison, the height of a standing 50<sup>th</sup> percentile female and male is 64 and 69 inches, respectively.

Clearly, stretching a rope at neck or head height, at a location where people may walk, run, or ride bicycles or motorcycles, especially at night, poses a clear hazard to public safety.

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#### Conspicuity, Camouflage, Depth Perception



### D20 Ethics in Forensic Engineering: Examples of Malpractice

*Darren Franck, MSME\*, Advanced Engineering Associates, Inc, Charleston, WV 25304; Harold Franck, MSEE\*, Advanced Engineering Associates, Inc, Charleston, WV 25304*

**Learning Overview:** The goal of this presentation is to address some of the issues detailed by the 2009 NAS Report, as well discuss as improvements in the state of forensic engineering.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting examples of ethical lapses in the field and discuss the benefits and efficacy of various mechanisms to enforce and encourage ethical behavior.

Unsupported claims, selective choice of evidence, wild allegations, untested conclusions, and confrontational language are often encountered in our legal system. These are usually disseminated by parties to a lawsuit or their legal advocates. In an ideal world, experts are brought into these cases to be an independent and disinterested arbiter of these disputes. Their conclusions should be based on facts and sound science. Sometimes the world proves less than ideal, as forensic experts have been known to engage in some of the worst behaviors of their clients. These behaviors represent serious ethical breaches that are largely unenforced and untested in the forensic engineering community.

Several cases will be presented that highlight examples of unethical behavior by forensic engineers. The motivations are frequently derived from a quest for money. Contingency is expressly prohibited by the canons of ethics promulgated by professional engineering organizations. Still, the certainty of a large windfall, or the promise of future work, can entice an expert to push the boundaries. Those that pass up long-term dependability for short-term gain often find no longevity in forensic engineering. Still, there are those that maintain credibility based on their name or connections. As the examples will show, their ethics (or lack thereof) is not in question. However, what actions, if any, should be taken about it?

One school of thought is that the “free market will mete it out” and that “the cream will rise to the top.” Clichés notwithstanding, there is truth to this viewpoint. Attorneys, clients, and members of a jury may not be experts in matters pertinent to engineering, but many have a considerable basis of knowledge. These laymen serve as bulwarks against experts that engage in sophistry. Our legal system is based on the idea that each party can present its case to a judge or jury. Certainly, an honest and competent expert should be able to present a better case than an unethical competitor. In this world view, no additional enforcement actions would be needed.

Another viewpoint is that unethical behavior by one is a stain on all. While it may be entertaining or satisfying to malign poor performance and blatant deceit by a competitor, the profession, as a whole, suffers when forensic engineers are seen as advocates. Standards, professional organizations, and licensing boards provide some layer of protection. However, groups charged with writing standards have largely been rendered incapable of recommending best practices, much less enforcing ethical behaviors. In fact, there has been a concerted effort by certain groups to evade and disrupt the development of standards in forensic engineering. Politics and personal relationships may limit the efficacy of complaints to a licensing board. Some engineering licensing boards have begun to mandate courses in ethics as a part of the renewal process. Within the legal system, *Daubert* challenges and their offsprings may protect a jury against unethical opinions that violate scientific principles.

Twenty-five years after *Daubert*, the state of forensic engineering and science has not sufficiently improved. The adversarial nature of the legal system in the United states promulgates the misuse of the role of experts. Ten years after the NAS Report, we have seen no significant improvement in forensic engineering. It is time for change, which requires the assistance from the legal community and the court system in the United States.

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#### Ethics, Forensic Engineering, NAS Report





### D21 The Role of the Technical Experts in Civil Law Countries: The Italian Case

Alessandro M. Ferrero, MSc\*, Politecnico di Milano, DEIB, Milano 20133, ITALY; Veronica Scotti, LLM, Politecnico Di Milano - DEIB, Milano 20133, ITALY

**Learning Overview:** After attending this presentation, attendees will better understand the role technical experts play in legal proceedings in countries with civil law systems. Italy is used as an example, since the manner in which technical experts act in its legal system is well representative of other European countries that adopt a civil law system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a look into the role played by technical experts in civil law systems, a role that is significantly different from the one played in common law systems. Attendees will gain a better awareness of what is expected from a technical expert and what the responsibilities are that he or she will face when serving as a technical expert in the different tasks that both criminal law and civil law proceedings assign them.

In both criminal and civil law proceedings, technical experts are appointed by the judge and serve as his or her technical alter ego, with the task of explaining to the judge the technical issues involved in the proceedings (fingerprint identification and DNA analysis in criminal law proceedings, and failure causes, defect identification, and compliance to standards in litigations).<sup>1-3</sup> Different than common law systems, the technical experts, being appointed by the judges, do not act as witnesses and face a different responsibility.<sup>4</sup> If, in rendering their expertise, they make serious technical mistakes that may mislead the judge, they can be charged with the crime of false appraisal that, in Italy, can be sanctioned with up to six years imprisonment.

Moreover, the parties (litigants in litigations, defendant and prosecutor in criminal law proceedings) can appoint their own technical experts that represent, from the sole technical point of view, the parties in every step of the technical expert activity and interact with the judge in an attempt to highlight the technical point of view of the party they represent.

The judge's technical expert produces, usually, a written report that, when related to litigations, must mandatorily include the observation(s) made by the parties, if submitted.

In general, the report submitted by the judge's technical expert represents the technical basis on which the judge makes his or her decision.

This presentation develops the above points, showing the different details of the role and responsibilities of the technical experts, both those appointed by the judges and those appointed by the parties.

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#### Technical Experts, Civil Law Systems, Italy



### D22 What to Do When a Client’s “Pet Theory” Conflicts With Good Forensic Science Practice

David R. Bosch, PhD\*, Phoenix, AZ 85028; Mark C. Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM 87015

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**Learning Overview:** The goal of this presentation is to examine several scenarios in which conflicting “pet theory” situations have occurred between forensic scientists and attorneys. The various potential outcomes and solutions for resolving such conflicts are discussed so forensic experts may gain some insight into how to prevent such problems either before they occur or to minimize the damage once such situations have happened.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating to forensic engineers, scientists, criminologists, attorneys, and other professionals involved in either civil or criminal litigation the need to be aware of these potential conflicts, as well as ways to recognize and prevent significant problems before they cause damage to either the case being investigated, and/or to the career of the forensic expert.

Many forensic scientists and engineers work with attorneys as consulting experts. There are times when a plaintiff or defense attorney client, prosecutor, police investigator, or others in the legal system develop a pet theory about a case. Such theories may be based on economic, political, prejudicial, or other reasons. Sometimes they are based merely on ignorance or misinformation. There may be situations when the pet theory can be in direct conflict with the laws of physics, factual evidence, and/or common sense. This can present significant problems to an objective, honest, ethical forensic professional who is called upon to investigate and analyze such a case.

In the engineering sciences, the First Canon of Ethics states: “One’s First Duty is to the Public.” This also means the forensic expert should be objective, truthful, and only render observations and opinions within their areas of expertise that are based on facts and proven, reasonable, scientific evidence.

Therein lies a common potential conflict between the “objective forensic scientist” who should have no personal or financial interest in the ultimate outcome of a case, and the “attorney advocate” who intends to win for his client, and often be compensated monetarily, by whatever legal means are available. This can become especially difficult if the forensic scientist is being paid by an attorney who is more interested in finding support for a pet theory that may be unsupported by anything resembling good science, than in following whatever path the evidence takes based on the expertise and scientific method utilized by the forensic scientist.

The foregoing difficult situation becomes even more strained when considering *Daubert* and other challenges to an expert’s scientific method and opinions. The expert’s career can often hang in the balance.

Attorneys may promise to support objective testing and other research to support an expert’s work and opinions, only to withdraw such support after the expert has been retained and begun investigating the case. This can have serious repercussions if the expert is prevented from establishing scientific fact that clearly supports the theories or goals of the client.

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#### Forensic Science, Pet Theories, Scientific and Engineering Ethics

## D23 The Potential Conflict Between Forensic Science and Certain Legal Approaches in Litigation

Kenneth J. Saczalski, PhD, Newport Beach, CA 92661; Mark C. Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM 87015; Todd Saczalski, BSMET, TKS Consulting, Inc, Colorado Springs, CO, CO 80907

**Learning Overview:** This goal of this presentation is to address the potential conflict between forensic scientific investigation, testing, and analysis versus various “legal approaches” sometimes suggested by attorneys in litigation cases. Those involved in the forensic sciences must be aware that the ethical and scientific demands must be properly met rather than altering investigative and test methods to simply present a case per an attorney’s preference.

**Impact on the Forensic Science Community:** This presentation will impact forensic scientists and engineers who must interact with attorneys and judges in either civil or criminal litigation and who are subject to *Daubert* and *voir dire* challenges by ensuring that the appropriate scientific and ethical standards are met regardless of how those in the legal profession wish to proceed with a case.

“Consumer expectation” is that vehicle safety systems protect motorists in 33mph–39mph Change of Velocity (Delta V) frontal impacts, and seats/belts should not fail in similar rear collisions. Study-conducted National Highway Traffic Safety Administration (NHTSA) rear crash tests of 15mph–20mph Delta V caused belted adult surrogates to catapult headfirst into rear survival space as front seatbacks collapsed, and restraints slackened, crushing rear-seated child surrogates. (For reference, 50 percentile of males are 69" tall, 167-pound instrumented crash test dummies per Code of Federal Regulations Title 49, Part 572 and 95 percentile of males are 74" tall, 227-pounds, and 5 percentile of females are 60" tall and 100 pounds. 6-year-old child dummies weigh 50 pounds.) These “unexpected” hazards result from no rear-impact, occupant-protection requirements and NHTSA’s admittedly “flawed and inadequate” Federal Motor Vehicle Safety Standard (FMVSS) 207 only requiring 275 pounds seat strength.<sup>1</sup> Quasi-static and dynamic crash tests for this study comparing weak Original Equipment Manufacturer (OEM) and safer, inexpensive alternative design seats consistently demonstrate predictable, preventable dangers. In addition to normal forensic science demands, this case study involved conflicts between study-recommended scientific tests and non-instrumented “demonstrations” requested by legal clients for “consumer expectation” approaches sometimes used in litigation.

In a 33mph–35mph Delta V Sports Utility Vehicle (SUV) rear impact, the 235-pound driver’s seatback with 1,960 pounds static load capacity predictably collapsed rearward, slackening vehicle-anchored belts, allowing headfirst loading into the Belt-Integrated center rear Seat (BIS), resulting in quadriplegia. FMVSS 210 requires that torso and lap belts withstand a minimum 3,000 pounds torso and 3,000 pounds pelvic frontal load; static tests of rear BIS demonstrated seatback strength of over 4500 pounds rearward upper torso load capacity.

Two identical 35mph Delta V dynamic crash tests compared a belted 235-pound surrogate in an OEM seat with 1,960 pounds strength versus unbelted in a second row BIS with 4,500 pounds strength (Fig 1.)

Test 1 was a “restrained” surrogate in an OEM seat with 13.9 maximum “G” resistance. (141-pound upper torso inertia force exceeding 13.9 G’s will collapse the seat and allow the restrained driver to ramp out of slackened vehicle-mounted restraints, consistent with this real-world case.)

Test 2 was an “unrestrained” surrogate in a second row BIS with 31.9 G resistance to occupant torso loads, which was 230% more resistant to crash forces than the OEM seat, while retaining similar seat track/seatback functionality, also providing much greater occupant protection, and could fit the driver floor pan and operating space.

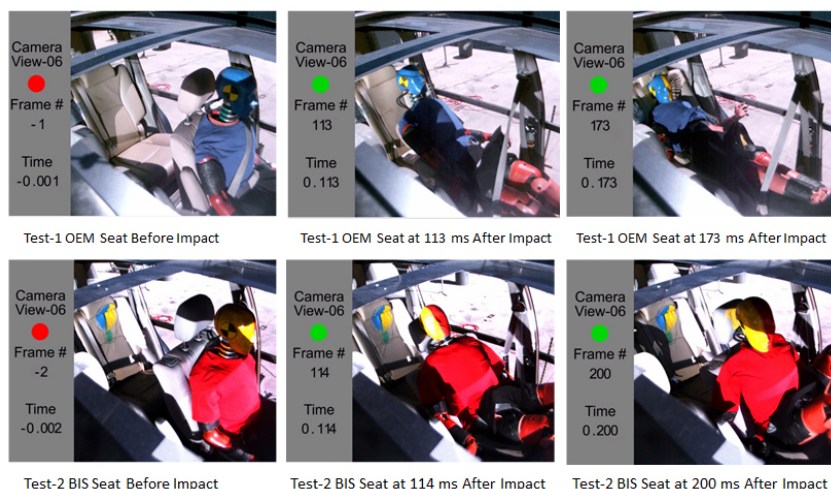


Figure-1: Video Clip Images Comparing Surrogate Maximum Rearward kinematics for Tests 1 and 2.



These tests demonstrated how “consumer expectation” tests should be run to scientifically prove, to a reasonable degree of engineering certainty, that: the belted surrogate in the weaker OEM seat experienced head injury ten times higher and neck injury 36 times higher than the unbelted surrogate in the BIS. These injury risks are consistent with paralyzing quadriplegia injuries to the actual driver; and (2) the unbelted surrogate in the stronger rear BIS had head and neck injury risk measures well below the injury thresholds acceptable by NHTSA, proving the BIS, even without belt use, gave far safer protection than the OEM seat and belts.<sup>2</sup>

The foregoing demonstrates a reliable method for clearly presenting scientifically verifiable evidence to consumers validating “consumer expectations” of what should or should not occur when a primary “safety system,” like the seat and belt restraints, fail to “function as expected” during foreseeable rear crashes.

Legal clients often misunderstand scientific aspects of a case and are concerned about testing costs of scientifically proving likely injury risk levels for the defective product and available alternative safer designs. Many challenges, such as *Daubert*, face forensic experts when legal clients restrict data gathering, scientific test-analysis comparisons, and alternate design approaches. Opposition experts may suggest that injury risks of alternate designs are no better than visual kinematics shown by the clients’ restrictive choice. Judges may allow such incomplete and unchallenged misinformation to be presented to a jury without allowing the forensic expert time to conduct scientific testing to provide a clearer picture of design comparisons. Non-scientific “consumer expectation” legal tactics using “visual demonstrations only” risks expert reputations and often costs far more than properly planned scientific testing.

INJURY MEASURES <span style="color: red;">(Red Measures Exceed IRV)</span>	OEM DRIVER SEAT Test 1	2 <sup>nd</sup> ROW BIS Test 2
<b>HIC</b>	<b>1,362.4</b> <span style="color: red;">(NHTSA Limit is 700)</span>	<b>133.6</b>
Ntf (tension-flex)	<b>0.15</b>	<b>0.21</b>
Nte (tension-ext)	<b>0.13</b>	<b>0.15</b>
Ncf (compression-flex)	<b>0.94</b>	<b>0.04</b>
Nce (compression-ext)	<b>0.95</b>	<b>0.01</b>
Neck Axial Compression (Newtons)	<b>- 6,036.2</b> <span style="color: red;">(NHTSA Limit is -4000N)</span>	<b>- 165.0</b>
Neck Axial Tension (Newtons)	<b>713.1</b>	<b>1,143.7</b>
Neck Flexion Torque (N-m)	<b>- 45.9</b>	<b>-27.6</b>
Neck Extension Torque (N-m)	<b>25.2</b>	<b>16.2</b>
Chest 3 msec (G's)	<b>29.8</b>	<b>24.4</b>

**Reference(s):**

1. Federal Motor Vehicle Safety Standard per 49 CFR, Part 571.
2. Author-tested 35mph rear-impact Delta velocity injury risk measures of 235 pound “belted” surrogate in weak OEM seat vs “unbelted” surrogate in second-row BIS from same vehicle. IRV=Injury Reference Values. HIC= Head Injury Criteria. Ntf, Nte, Nef, and Nce are acronyms for individual measures of the NHTSA neck injury criteria (All Injury Criteria per 49 CFR Part 571).

**Forensic Science, Testing, Litigation**



### D24 The Vision and Progress of the Organization of Scientific Area Committees (OSAC) Fire Debris and Explosives (FD&E) Analysis Subcommittee

Susan Seebode Hetzel, BA\*, S-E-A, Ltd, Columbus, OH 43229-1157

**Learning Overview:** The goal of this presentation is to inform attendees of the progress being made within the OSAC as it relates to FD&E analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating laboratories of the progress being made by the OSAC FD&E Subcommittee. The recommendations coming from the OSAC will impact all analysts and laboratories that perform FD&E analysis.

The OSAC purpose is to strengthen the nation's use of forensic science; it was formed as a response to the 2009 National Academy of Science (NAS) Report *Strengthening Forensic Science in the United States: A Path Forward*.<sup>1</sup> OSAC is divided into five Scientific Area Committees (SAC) which are currently further divided into 25 subcommittees. The FD&E Analysis Subcommittee is one of those 25 subcommittees and is part of the Chemistry/Instrumental Analysis SAC.

The main goal of all 25 subcommittees is to write consensus-based standards to be used by their discipline that are fit for purpose and based upon sound scientific principles. All of these standards must go through a Standards Development Organization (SDO), and the American Society for Testing and Materials (ASTM) is the SDO that will be used by the FD&E subcommittee.<sup>2</sup> There are already eight ASTM standards that relate specifically to fire debris and two that relate to explosives (smokeless powders). Most of these existing standards need a little tweaking to firm up their base, but some need a major overhaul in order to address the concerns that will dominate the future of forensic science.

The overall schemes (road maps of documents) that are envisioned for both fire debris and explosives will be presented and discussed. The proposed documents will include sample screening, sample preparation, instrumental analysis, data interpretation, reporting, analyst training, Quality Assurance/Quality Control (QA/QC) requirements, and method validation. Luckily, we do have the existing ASTM standards, and the work that was done previously by the Technical/Scientific Working Group for Fire and Explosions Analysis (T/SWGFEX) to use as starting points.<sup>3</sup> How far are we now in this process, and how much farther do we need to go?

With fire debris, we are farther along in the process, mostly because of the legacy ASTM standards that already existed; some for more than 30 years. However, are there ways that we can make it better? Can we tighten up the criteria so there is more consistency from analyst to analyst, and laboratory to laboratory? An overview of the vision the FD&E subcommittee has for the instrumental analysis and interpretation of fire debris, as well as the possibilities for the future of fire debris analysis, will be discussed.

With explosives analysis, there is a lot of work to be done. There are no currently existing consensus standards on how to approach the analysis of explosives. There are both intact explosives and post-blast analyses to consider. The previous work of T/SWGFEX, when available, is being used as a starting point upon which to build.

#### Reference(s):

- <sup>1</sup> Harry Edwards, Constantine Gatsonis. *Strengthening Forensic Science in the United States: A Path Forward*., Washington D.C., National Academic Press, 2009, <http://books.nap.edu/catalog/12589.html>.
- <sup>2</sup> All ASTM standards are published by ASTM International, West Conshohocken, PA. Available via <https://www.astm.org>. Accessed 21 June 2018.
- <sup>3</sup> SWGFEX.org. Accessed 31 July 2018.

#### Fire Debris, Explosives, OSAC



### D25 The Use of Toxicological Evidence in Fire Investigation

*Douglas J. Carpenter, MS\*, Combustion Sci & Eng, Inc, Columbia, MD 21045*

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**Learning Overview:** The goals of this presentation are to: (1) put forth a methodology for using toxicological data for the testing of hypotheses using the application of the Scientific Method to the investigation of fire and explosion incidents; and (2) provide examples of its use and discuss the limitations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new and reliable methodology, will show how it can be used and its value, and include a discussion of the limitations.

Scientifically reliable determinations in forensic investigations are predicated on the use of a scientifically reliable methodology. The methodology recommended by National Fire Protection Association (NFPA) 921, the *Guide for Fire and Explosion Investigations*, states the “standard of care associated with the forensic investigations of fire and explosion incidents is the Scientific Method.” NFPA 921 defines the Scientific Method as “The systematic pursuit of knowledge involving the recognition and definition of a problem; the collection of data through observation and experimentation; analysis of the data, the formulation, evaluation and testing of hypotheses; and, where possible, the selection of a final hypothesis.”

Recognizing that *data* is information that can be documented and verified, and *evidence* is *data* that is relevant and reliable in a specific context, *evidence* is the key to the reliable application of the Scientific Method to the investigation of fire and explosion incidents. Data for use as evidence using the Scientific Method may come in many forms. Fatal fires or fires that involve human injuries may provide such data and evidence. There is a direct connection between the conditions necessary for the production of toxic products of combustion in fires and the dose response of the uptake of the products of combustion in mammals. This direct connection of autopsy and injury data from victims of fire may provide the fire investigator with important, discriminant, scientific evidence to assist in the testing of hypotheses associated with the fire origin and *fire cause* determination.

Through the combined use of fire testing, fire modeling, and physiological modeling, the fire investigator may be able to test or further validate their origin and *fire cause* hypothesis(es), and other aspects of a fire incident based on the facts of the case and data collected during autopsy or hospital evaluation. As demonstrated in the case study, autopsy data and evidence were compared against carbon monoxide (CO) concentrations and temperature profiles for two competing origin and *fire cause* hypotheses. Only one of the fire scenarios produced toxicant doses and thermal conditions are uniquely consistent with the victim’s injuries. A discussion of the limitations of the use of autopsy data and identification of unique conditions with the fire dynamics of “locally under-ventilated” fires will be discussed. Ultimately, the evaluation of autopsy data in combination with the facts of the case and dynamics of the fire assisted in producing scientifically reliable origin and *fire cause* determination using the application of the Scientific Method.

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#### Toxicology, Fire, Investigations



### D26 Exceeding the Scope of Law Enforcement in Forensic Fire Investigation

S.B. Addison Larson, MS\*, Sherman, CT 06784

**Learning Overview:** After attending this presentation, attendees will understand the danger of law enforcement interference and the potential for unchecked bias if forensic science practitioners and researchers lose autonomy and independence to police agencies, particularly in reference to forensic fire investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by lending support to current ideas regarding the sources of cognitive bias and unregulated practice in forensic science, specifically if forensic functions are incorporated into law enforcement agencies without a controlling plan or oversight.

An example in Connecticut underscores the importance of maintaining independence and objectivity in the field of forensic fire investigation. In 2011, the Connecticut State Fire Marshal's Office restructured operations management for fire investigation by transferring the investigative division from under the auspices of Emergency Services, placing it under the purview of the Connecticut State Police (CSP). The division was rebranded as CSP Fire and Explosion Investigation Unit (FEIU) and has, since that time, assisted in excess of 300 fire investigations per year.<sup>1</sup>

Because the FEIU is a division within the CSP, all state fire investigators are now sworn law enforcement members; the FEIU does not employ any civilian or non-police investigators. Furthermore, the FEIU is mandated to investigate upon written complaint local fire marshals reported as incompetent or negligent.

The FEIU is not accredited, nor are they authorized to conduct any fire-related research; they have no standardized procedures or methods that relate to the broader fire science community. They are the only forensic section of the CSP that has not already begun the process of civilianizing. Forensic fire investigation, as defined in the National Fire Protection Association's publication *NFPA 921: Guide for Fire and Explosion Investigations*, is not included among the forensic science disciplines covered by the CT State Division of Scientific Services.<sup>2</sup> Current practice delegates to the CSP physical and administrative oversight of non-law enforcement personnel; allows police to insert themselves into objective public sector investigations; and fails to safeguard against or account for the potentiality of mistakes due to the unintentional or intentional bias of state troopers and witnesses.

The integration of forensic science into law enforcement, or the idea that a policing agency may also perform scientific analysis of evidence, poses the danger of a "systemic pro-prosecution bias [and] is a function of the same fairly obvious psychological concepts of cognitive bias."<sup>3</sup> The scope of a police investigation is not to interpret evidence, but to use forensic reports provided by an impartial certified fire investigator or a fire marshal to develop a criminal case. Police involvement at a fire scene is unnecessary, unless the fire marshal classifies the fire as incendiary.

The information presented pertains to the field of fire and arson investigation, but may provide insight on a small scale to current issues within the forensic science community, especially in light of the passive disbandment of the independent forensics panel which partnered with the United States Department Of Justice (DOJ) and the National Institute of Standards and Technology (NIST) after the release of the National Academy of Sciences (NAS) Report, *Strengthening Forensic Science in the United States: A Path Forward* (2009).<sup>4</sup> Recommendations within the NAS Report include the creation of an independent federal entity to oversee the field, and the removal of crime laboratories from the administrative control of law enforcement agencies.<sup>4</sup>

#### Reference(s):

1. Dept. of Emergency Services and Public Protection, *At a Glance*, 2011.
2. National Fire Protection Association. Technical Committee on Fire Investigations. *NFPA 921, Guide for Fire and Explosion Investigations*, National Fire Protection Association (2017).
3. Donald E. Sheldon. Closing the Gate on Biased Expert Testimony: The Judicial Perspective (Bias in Forensics - Examining the Sources and Impacts of Bias on Perceptual and Cognitive Judgments Made by Forensic Experts). *Proceedings of the American Academy of Forensic Sciences*, 66<sup>th</sup> Annual Scientific Meeting, Seattle, WA. 2014.
4. National Research Council. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press (2009) <https://doi.org/10.17226/12589>.

#### Prosecutorial Bias, Forensic Fire Investigation, Arson Investigation



### D27 Heavy Truck Fuel-Fed Fires: Predictable Fuel Leakage, Ignition Sources, Fuel Tank Placement Hazards, and Safer Alternative Designs

Mark C. Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM 87015; David R. Bosch, PhD\*, Phoenix, AZ 85028; Kenneth J. Saczalski, PhD, Newport Beach, CA 92661

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**Learning Overview:** After attending this presentation, attendees will be equipped to recognize and analyze heavy truck fuel system failures and fires, understand the survivability of such fires, and egress/escape from burning vehicles. Current designs will be analyzed and compared to safer alternative designs for fuel tank location, tank materials, tank liners and filler materials, check valves, impact guarding, etc.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community in terms of the forensic aspects of traffic accident investigation and reconstruction or fire investigation.

Some school buses and medium trucks utilize gasoline or propane-powered engines, which typically utilize fuel tanks located inside the frame rails and/or which are guarded against collision damage. A small but growing number of heavy trucks or large buses utilize compressed or liquified natural gas, with most fuel tanks mounted behind the cab, but with a few mounted outside the frame rail. Most medium and heavy trucks worldwide tend to be powered by diesel engines. The placement of diesel fuel tanks and any guarding varies widely depending on the size of the truck as well as the safety regulations in the country of operation.

In many areas of Europe and other countries, such heavy truck diesel fuel systems are guarded for impact protection. However, the heaviest Class 7 and 8 trucks and vocational vehicles operated in the United States typically utilize large-volume unguarded side-saddle type fuel tanks attached outside one or both frame rails, usually in close proximity to the cab. Many such fuel tanks incorporate steps to allow ingress and egress from driver and passenger seating areas. Many such exposed fuel tanks are adjacent to unguarded batteries and cables mounted outside the frame rails. This creates an extraordinary proximity of exposed fuel and ignition sources that are subject to collisions by other vehicles and fixed objects, which also happen to be adjacent to vehicle occupants. Failures of such exposed fuel systems and ignition sources has often occurred at low velocities, with resulting dangerous fuel leakage and fires. Egress and escape are often limited to doors or windows located directly above such vulnerable fuel tanks.

The exposed location of such heavy truck fuel tanks clearly violates many long-established principles of reasonably safe vehicle fuel system design for passenger vehicles and light trucks. Crashworthy fuel system designs have been determined by crash testing research that discovered dangerous flaws in fuel tank location and materials, and this has been published since at least the mid-1960s. One author of this study participated in the National Highway Transportation Safety Administration vehicle fuel system safety research in the 1970s and 1980s per Federal Motor Vehicle Safety Standard 301, as well as defect investigation crash tests on the Ford® Pinto®, General Motors® side-saddle tank pickups, and several other vehicles, that led to safety recalls. Another author consulted on vehicle fuel system safety, including the Ford® Pinto®, for the Department Of Transportation (DOT) and the Department of Defense. The Federal Motor Vehicle Safety Standard (FMVSS), including 301, tend to be significantly less robust or non-existent for larger vehicles with Gross Vehicle Weight Rating (GVWR) over 10,000 pounds.

Among the “justifications” for such obviously unsafe heavy truck fuel system designs is the significantly greater size and weight of medium and heavy trucks relative to passenger vehicles, as well as the supposedly lower flammability of diesel fuel compared to gasoline. The extremely dangerous sidesaddle fuel tank design of the General Motors® 1973–87 pickups was ample proof of how such placement outside the frame rail was vulnerable to failure in relatively minor collisions with other vehicles or fixed objects. Some of the “justifications” for such hazardous heavy truck fuel systems are based more on incorrect assumptions, myths, and economic/political considerations rather than any reasonable safety engineering.

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#### Heavy Truck Fires, Vehicle Fuel Systems, Crash Hazards





### D28 Electrical Homicide or Not?

*Helmut G. Brosz, BASc\*, Forensic Science International Group, Markham, ON L3P 3P2, CANADA*

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**Learning Overview:** After attending this presentation, attendees will understand that forensic engineers can play an important role in reconstructing the scene of an electrocution. These reconstructions assist criminalists in determining if a death could have been a criminal act that was perpetrated with the use of electricity, either intentionally (electrical homicide) or unintentionally (negligent homicide).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to an improved performance of criminalists and others involved in an investigation of a suspected electrical homicide by availing themselves of forensic engineers and their expertise.

In this presentation, cases will be presented in which the cause of death is electrocution. Electrocution generally means that current has passed through the heart and death ensued. Through investigation, one may discover whether this death was caused intentionally, by criminal negligence, or other causes.

Electrical injuries can be multifaceted, and in many cases can cause devastating trauma to the victim's body or no visible trauma at all. The severity of electrical trauma can range from gross loss of limb and tissue due to sustained high voltage and high current contact. Low voltage and low current contact may not leave any marks on the body, depending on contact area, duration, and other environmental factors. Since most electrical injuries involve marks on the body, there can be challenges in accurately assessing the pathway(s) of current and the modes and circumstances of contact. When an electrocution is being examined, investigators must inspect the scene and the body before concluding.

In some cases, it can be difficult to determine whether the incident was deliberate, an accident, or if an electrocution occurred due to lack of evidence. For example, if a victim were to be electrocuted by fallen electrical wires, there are many possibilities of what caused the wires to fall in the first place. One possible situation is if an electrical worker installs wires in such a manner that they could foreseeably cause an electrocution, then such a case could be considered negligent homicide or manslaughter. Another situation is that the electrical system was sabotaged in some way and, if so, this would be intentional electrical homicide. The incident could also be the fault of the victim, such as electrical suicide or misadventure. Natural causes, such as floods, winds or a storm bringing down power lines and poles, as well as lightning, are other causes of electrocution.

When investigators examine the scene of an electrocution, they cannot afford to make any assumptions. Although some instances may appear to have a clear cause, all possibilities must be taken into consideration first, with the assistance of a forensic electrical engineer and their expertise.

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#### **Electrocution, Homicide, Electrical Injury**



### D29 Sabotaged Electrical Grid: Worse Than 9/11?

*Helmut G. Brosz, BASc\*, Forensic Science International Group, Markham, ON L3P 3P2, CANADA*

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**Learning Overview:** After attending this presentation, attendees will see that forensic engineers can play an important role in determining if criminal sabotage or an accidental sequence of events has occurred. The by-product of such an investigation is often an increase in the reliability of the electrical grid.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to an improved performance of criminalists and others involved in an investigation and postmortem of an intentional electrical grid failure by availing themselves of forensic electrical engineers.

This presentation is based on 50+ years of experience as a hands-on Electrical Engineer working for and with electric utilities nationally and internationally, and having caused accidental blackouts, experienced blackouts, investigating many blackouts, as well as breakdowns and failures of electrical equipment, including transmission lines, distribution lines, major power transformers, generators, and substations.

In addition, the study of major blackouts in the United States, Canada, Russia, Australia, Greece, Venezuela, Pakistan, and Jamaica, just to name a few, has caused one to pause and reflect on the dire consequences of a lack of electricity in our electricity-dependent world.

For example, on a small scale, an extended power failure in a high-rise condominium brings about the putrefaction of refrigerator contents, lack of circulating water, loss of heat and cooling, elevators, communication, and eventual evacuation. If we now scale this up to a city-wide outage, food stores will also close, gas stations will not pump fuel, the water supply will eventually dry up, police and emergency services will diminish, hospitals, banks, and credit card services will stop, cash will be king until barter and robbery takes hold, and so on. If we now scale this up to a region-wide outage affecting seven states and one province, as on Aug 14, 2003, then the outage effect really gains traction. The World Trade Center attack of 9/11 killed and injured several thousand people; a power failure of one week could kill as many, and a power failure lasting one month and longer will bring our society back to primordial behavior, much to the satisfaction of the perpetrators. The forecast will be left to psychologists and others in this field.

The criminalists and other investigators will be busy trying to survive.

So far, it is believed that most outages have been accidental or, let's say, not deliberate. However, there is a fine line between those two terms. "Accidents" can be staged and created. Without turning this presentation into a "how to," let it be said that we are very vulnerable today despite the various protection and backup systems that have been put in place after each blackout. These vulnerabilities still need to be addressed.

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#### Electrical Grid, Sabotage, Blackout



### D30 American Association for the Advancement of Science (AAAS) Forensic Science Assessments: A Quality and Gap Analysis of Fire Investigation

Jose R. Almirall, PhD\*, Florida International University, Miami, FL 33199

**Learning Overview:** After attending this presentation, attendees will be familiar with the July 2017 report published by AAAS on the quality and gap analysis in the field of fire investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing conclusions and recommendations reported by the AAAS fire investigation working group.

The AAAS, the world's largest scientific society, recently commissioned a quality and gap analysis on the topic of fire investigation. The AAAS staff and a working group composed of a fire investigator and academic scientists reviewed the current practice and available scientific literature in the field and reported their findings in July 2017.<sup>1</sup> The working group was asked to identify gaps in the body of knowledge within fire investigation and make recommendations for future research that would fill the gaps and "inspire greater confidence in the criminal justice system." The report was divided into two parts, fire scene investigation and fire debris analysis, and includes conclusions and recommendations related to the different aspects of each of these methodologies. Fire scene investigation covers the topics of origin and cause determination, locating ILRs in fire debris, reliability and validity, cognitive bias and education, certification and experience. The fire debris analysis section of the report covers topics that include potential improvements in locating and analysis of ILRs at the scene using more advanced analytical chemistry tools, applying analytical methods in the laboratory, advances in the standardization of analytical practice, challenges in the analysis of Ignitable Liquid Residues (ILRs), including the interpretation of the analytical data collected from fire debris analysis and, finally, reporting language.

The report is formatted to include specific recommendations for both the fire scene investigation portion and the fire debris analysis portion. The report also includes conclusions drawn from the review of the available literature to aid policy makers and funding agencies in developing a strategy to facilitate research where gaps in knowledge are identified. The fire scene investigation portion of the report includes recommendations in the areas of origin and cause determinations, locating ILRs in fire debris, reliability and validity, cognitive bias and education, certification, and experience.

The recommendations and conclusions drawn from the fire debris analysis portion of the report include improving the identification and, hence, collection of ILRs in the field through the use of portable instrumentation, trained canines, and other means, improving the application of analytical chemistry tools in the laboratory (sampling, extraction and analysis), addressing the current challenges in analyzing ILRs, such as the classification of ILRs in a standardized way, a study on the limitations on the current methods, and the need to know the performance of the current standard methods in use by the fire debris analysis community (global and local error rates).

#### Reference(s):

- <sup>1</sup>. José Almirall, Hal Arkes, John Lentini, Frederick Mowrer and Janusz Pawliszyn. Fire Investigation. *Forensic Science Assessments: A Quality and Gap Analysis*. 7/11/2017. <https://www.aaas.org/report/fire-investigation>.

#### Fire Investigation, Fire Scene Investigation, Fire Debris Analysis



### D31 A Case Report on a Cable Car Stoppage Accident Caused by Wild Mice

*Youngsu Kong\*, Department of Physical Engineering in Gwangju, Jangseong, Republic of Korea; Jaehak Lee, PhD, Jangseong-gun, Korea, SOUTH KOREA; Gie-tae Lee, MS, National Forensic Service, Gwangju Institute, Jangseong-gun, SOUTH KOREA; Jae-mo Goh, PhD, National Forensic Service, Wonju-si, Gangwon-do 26460, SOUTH KOREA; Nam-Kyu Park, PhD, National Forensic Service, Wonju, SOUTH KOREA*

**Learning Overview:** After attending this presentation, attendees will be informed about a special case of a cable car stoppage accident caused by wild mice and will thus be informed of more possibilities to be considered when investigating cable car accidents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a case report for a non-typical case of a cable car stoppage accident caused by wild mice.

With technology development and urbanization, accidents caused by wild mice are gradually disappearing in South Korea. Nonetheless, buildings in the countryside and mountains of a famous place occasionally have accidents caused by wild mice. In one case, a cable car that was normally running suddenly stopped due to wild mice. On November 5, 2015, at Duryun Mountain in Haenam, South Korea, the cable car, which was operating normally with passengers, was suddenly stopped by a power failure.

The site of the accident consisted of a cable car, a cable car platform, a machine room, and a cab. The machine room was equipped with the main power and the Automatic Transfer Switch (ATS) panel, a generator to supply spare power, a motor to drive the cable car, and a wire rope pulley. The ATS installed on the ATS panel was partially destroyed due to electrical fault. After replacing the ATS, the operation test for the cable car system was performed and the cable car system operated normally. The ATS operates as follows: the ATS is in contact with the main power terminal when the cable car is operating normally, but it automatically contacts the backup power terminal of the ATS when there is a problem with the main power supply of the cable car. Through scene investigation, it was found that the cause of sudden stoppage of the cable car was the ATS breakage. As a result of the inspection of the ATS, the main and backup power indicators of the ATS were both on and the ATS conversion switch had been moved to the backup power side. Two mice bodies were found on the R phase and the S phase between the main and backup power terminals of the ATS, respectively. Many electric arc marks were observed on the R phase, the S phase, and the T phase of the main power and standby power terminals.

According to the results of the scene investigation and ATS inspection, the cause of the cable car accident was the breakdown of the insulation due to the intrusion of the wild mice between the R phase and the S phase of the main power and standby power terminals of the ATS. Such a situation, with buildings in rural and mountainous areas of famous places, can still cause accidents due to wild mice. This case report can be used as a reference for future similar accidents.

#### Wild Mice, Automatic Transfer Switch (ATS), Cable Car



### D32 Data Center Failures, Losses, and Litigation

*Helmut G. Brosz, BSc\*, Forensic Science International Group, Markham, ON L3P 3P2, CANADA; Ralph A. Ruffolo, BSc\*, Clearwater, FL 33766*

**Learning Overview:** After attending this presentation, attendees will understand some of the common causes of data center failures, which often lead to litigation, and preventive remedies of such failures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping experts to identify in data center failures: (1) cause; (2) origin; (3) responsibility; (4) preventability; and (5) how to assist with insurance and legal claims.

Data centers require reliable electrical power, Uninterruptable Power Supply (UPS) systems, batteries, back-up generators with reliable and sufficient fuel, cooling systems, transfer switches, and switchgears, including reliable protection systems.

**Case 1:** Water pipe and valve failures-flooding—Water cooling and circulating systems are required to withstand certain pressures, temperatures, and flow velocities in order to keep electronic equipment cooled. Pressure tests are usually conducted soon after installation. However, in-plant inspections of pipes, shut-off valves, and fittings before they are shipped to a site are seldom performed. Inspection in a manufacturer's plant can go a long way toward catching defects before shipment.

**Case 2:** Back up diesel engine/generator failures—In some cases, improperly manufactured oil filters have allowed metal particles to bypass such filters and damage bearings. In other cases, defective diesel engine fuel injectors were found to leak and cause explosions in the exhaust system. Most emergency diesel generators are turbocharged and are sometimes not manufactured to tight tolerances. As a result, they have been known to fail and start lubricating fires since they operate at very high temperatures. Fuel supplies have been found to contain water and cause malfunction of diesel engines. In-plant inspections and review of specifications of diesel engines during manufacture are an important prevention remedy.

**Case 3:** Electrical protection failures—In large data centers, 480-volt switchgears usually contain overcurrent relays only. Directional overcurrent relays are often not used but should be used. Also, in many instances fuses and relays are not properly coordinated, thus causing larger outages in a system than necessary.

**Case 4:** Small UPS failures start fires—Small under-the-desk UPS units have been known to fail due to defective solder joints.

**Case 5:** 480-volt switchgear/4,000-amp circuit breaker failure and Automatic Transfer Switch (ATS) failures—Drawout circuit breakers require current-carrying contact fingers that can carry not only rated current but also fault current for a prescribed time, otherwise they may overheat, arc, and fail. Finger contacts are often improperly machined and thus have insufficient contact surface. There may also be insufficient contact pressure and misalignment. Corrosion on contact surfaces can also contribute to overheating.

**Prevention Remedies:** In-plant inspection as well thermography at various intervals during the testing and commissioning process can go a long way toward failure prevention.

In an actual case, a 1,600-amp ATS failed for the following reasons: the racking assembly roller was bent out of position by almost one-half inch, so that upon racking in the switch into its connected and operating position, the main finger contacts did not properly mate with the fixed plated copper stabs of the switchgear. Thus, only a few fingers in the contact cluster made contact. This had the detrimental effect of de-rating the current carrying capacity of the finger cluster-stab contact. Overheating over a period of time led to arcing within the contact, which melted the remaining contact surfaces. This was followed by an explosion where conducting copper vapor was produced which then created a phase to phase-to-ground fault. If the prevention remedies set out above had been followed, this loss would not have occurred.

#### Data Center, Failure, Litigation



### D33 Propane Explosions: The Result of Failed Water Heater Gas Control Valves—Manufacturing Flaws Brought on by Design Changes by an Outsourcer

Tim G. Dunn, MS\*, Dunn Laboratories, Inc, Roswell, GA 30076

**Learning Overview:** After attending this presentation, attendees will be familiar with some basic principles on the safety features of gas control valves, as well as how changes in valve component, by a third-party vendor, can lead to gas fires and explosions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how unilateral design changes by outsourcing vendors of critical subcomponent parts, unknown to the control valve manufacturer, can affect the product's overall safety.

Following two propane explosions, it was concluded that the source of the fugitive gas was the failure of water heater control valves. Additional incidents came to light during these investigations. All shared these common threads: (1) out of gas conditions or a pilot outage. The victims were burned while trying to relight the appliance pilot; (2) same brand water heater control valves; (3) the failure of the valve's internal safety magnet to properly close off flow following the gas outages; (4) the same basic safety magnets used with gas appliance control valves since the 1930s; and (5) a common mode of failure: a peeling off of the rubber seat from the top mount on the valve's safety magnet, such that once off, the seat would not provide closure. The consequence being that on the occasions of an interruption of gas service or pilot flame outage, this insidious failure would permit raw (unburned) gas flow—absent a lit pilot light—to the constant (or standby pilot), and when called on (i.e., tank water temperature dropped below the desired set point), a much larger discharge of fugitive gas through the main burner.

In recent years, the safety magnet had been supplied to the valve manufacturer by an outsourcing (third-party) vendor: unbeknownst to the valve manufacturer at the time, this third-party vendor had changed (reduced) the dimensions of the magnet's top metal tip (referred to as a nesting button), where the donut-shaped rubber seat was nested, thus leaving the seat susceptible to being peeled off.

Even as more incidents occurred, the valve manufacturer maintained inexplicably that the separation (peeling off) of the seat from its magnet mount was a direct result of overpressure conditions. However, testing of the subject (incident) pressure regulators and later, high pressure testing in lab conditions, disproved this failure mode.

Residing within the valve's inlet chamber, the primary purpose of this safety magnet is to shut down gas flow to both pilot and main burners in the event of pilot flame outage. A failure of this safety magnet will allow unburned or raw gas to flow to the appliance burners in absence of a lit pilot. A thermocouple, with its head impinging upon the pilot flame, will feed a small (millivolt) electrical current into the safety magnet, sufficient to energize the magnet. Following the pilot lighting steps, the valve's reset button is manually depressed, which draws the safety magnet's rubber seal down and away from its metal closure opening, which in turn pushes the attached spring-loaded stem so that its mating plate bottoms-out against the magnet; once the pilot flame is established and the thermocouple is generating current, the energized magnet should hold the mating plate down, thus permitting gas (or gas flow) into the other valve chambers.

In these incidents, the peeling off of the rubber seat would have occurred during previous pilot lightings, when the control valve was operated as described: while properly aligned and in place, the affinity (or sticking) of the closed rubber seat to its metal closure was the cause of the failure. This failure only occurred in the more recently manufacture valves, specifically magnets provided by the third-party vendor with reduced-sized top (nesting) buttons.

Review of the valve manufacturer's documents and drawings of the safety magnet assembly reflected an inexplicable change in dimension of the top (nesting) button. The 10/20/94-dated drawing depicts a 0.472-inch diameter button, while the 2/23/02 drawing reflects a 0.421-inch diameter, with no engineering change order. Again, measurements of the failed control valves revealed safety magnets with 0.42-inch diameter top buttons. Related to the alleged overpressure theory, in the drawing, the rubber seats are flush against the base metal disk: in other words, there is no excess rubber hanging off the base disk and exposed to the directional gas flow, whether it is nominal or high pressure.

In deposition testimony, the valve manufacturer's senior principal engineer explained that in previous drawings, the diameter of the top (nesting) button was incorrect: the outsourcer had always supplied safety magnets with a 0.421-inch diameter top (nesting) button.

The relevant section of deposition testimony follows: Question: Why? Answer: We were told by the outsourcing vendor that was not (1) correct; that the dimension had never been .472. It had (2) always been .421. So, it was to update our drawing, and, (3) correct it to what it should have been. Question: Okay. So ... so, in other words, the .472 never existed. Answer: That's correct. That's correct.

In contradiction to this testimony, notwithstanding previous drawings, the earlier vintage of same brand control valves, both had top (nesting) buttons with the larger (0.472) diameters. An unmarked magnet (possibly not from this outsourcer) with a red rubber seat has a date code 9427, while the other, safety magnet (supplied by the same third-party vendor) has a date code 9405.

This presentation will briefly discuss the basic design of appliance gas valves, the critical role that safety magnets play, relevant aspects of the incident investigations and testing, as well as the conclusions reached.

After this presentation, attendees will be familiar with how subtle changes in the design of a subcomponent can affect the integrity of a key component of a pilot-ignited gas appliance.

#### Propane Explosion, Water Heater, Control Valves

## D34 An Explosion of a Primitive Industrial Oven in India: A Forensic Analysis

Jatin Bodwal, MD\*, Deen Dayal Upadhyay Hospital, New Delhi 110064, INDIA; Anil Aggrawal, MD, Maulana Azad Medical College, New Delhi 110002, INDIA

**Learning Overview:** After attending this presentation, attendees will understand the workings of a primitive type of industrial oven and the various causes behind its explosion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the workings of a primitive type of industrial oven and the various causes behind its explosion.

The explosion of an industrial oven results in an extremely devastating outcome, which usually claim the lives of people who unfortunately are present in proximity of the blast site.

Invariably, inadequately trained and impoverished people who migrate to New Delhi (the capital city of India) from poor states such as Bihar and Uttar Pradesh are the ones who risk their lives every day while working in a factory where these primitive industrial ovens operate.

**Working of an industrial oven:** (A seven-minute video will be shown to explain the working of a primitive industrial oven.) A typical industrial oven has two doors, one entry and one exit. Both the entry and exit doors can be used interchangeably (Figure 1). Inside this oven is chamber in which there are two parallel rods that are placed at the bottom of the chamber. These rods have multiple holes that light up due to the supply of Liquefied Petroleum Gas (LPG) and generate a high degree of temperature inside the chamber. The temperature monitoring is done by a thermometer that hangs on the ceiling. The usual temperature generated for optimum working of the oven is ranges between 120°C to 170°C. Surprisingly the source from which the oven get its heat is a typical household LPG cylinder (Figure 2). The sliding fashion assembly of metallic plates or other circular-shaped table fan tops or ceiling fan tops go inside the chamber and both the doors are closed (Figure 3). The assembly of metallic plates is usually arranged in the row. This assembly of objects remain inside the chamber for about 15 to 30 minutes. The purpose of this oven is to get the metallic paint firmly stuck to the surface of these objects.

Presented here is the case of a worker who died on the spot due to an oven blast while working. The main reason that can be attributed to the blast will be thoroughly discussed. In this instance, a worker was working in the factory when a fellow worker rushed to the oven when they heard a loud blast sound. They found a worker lying on the floor, injured. They rushed him to hospital, but he was declared dead on arrival.

At autopsy, the deceased was found to have suffered both burn injuries in the form of superficial burn and blunt injuries, in the form of a fracture (Figures 4 and 5). Death was attributed to the head injury and multiple fractures.

The chief reason for an oven blast is an extremely high temperature inadvertently generated if a worker forgets to switch off the supply of LPG gas or open the gates. Most of the modern sophisticated industrial ovens are equipped with safety valves, which function to alleviate excess heat. If the valve become dysfunctional owing to some clogged pipe or the oven is not equipped with safety valves, then excess temperature causes an oven blast.

India is a developing country where ill- or inadequately trained youths jump into factory work where these primitive types of oven are being used, without knowing the potential hazards of their job. Safety gears, which should be essential while working, are hardly ever provided by the employer to these poor workers. Only certified persons who take formal training from recognized institutes for working in factory should be hired.



Fig. 1: Door of industrial oven unit



Fig. 2: Household LPG cylinder



Fig. 3: Assembly of metallic objects



Fig. 4: Burn injuries on the body of deceased



Fig. 5: Fracture of the humerus bone

### Explosion, Industrial Oven, India

### D35 A Framework for the Cost Evaluation of Deforestation Based on the Habitat Equivalency Analysis (HEA) Principle

David Domingues Pavanelli, PhD\*, Brazilian Federal Police, São Paulo 05038-090, BRAZIL; Nickolaos Voulvoulis, PhD, Imperial College London, London, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will understand the role of financial evaluations in Brazilian environmental forensic reports and the methods used by forensic experts to calculate the total economic value of environmental damages.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the potential of the framework to inform the forensic science community, improving the ability to evaluate interim losses, perpetual damages, and propose better approximations of the total economic value of deforestation.

Under Brazilian law, environmental damages are considered criminal offenses. During the criminal process, environmental forensic experts normally are expected to appraise the damage to set bail, a fine, and the minimum value for its repair. The final financial damage valuation is defined during a civil process and trial, in line with the valuation described in the criminal process.

Deforestation is a typical Brazilian environmental crime. Environmental forensic experts typically evaluate the damage caused by deforestation using the commercial value of the wood extracted plus the cost of reforestation. However, many non-marketable ecosystem services provided by forests are rarely considered in this process. Well-described examples of these include erosion control, water supply, nutrient cycling, pollination, biological control, and many other ecosystem services. Not including these services in valuations of environmental damages is misleading public perception of the true total environmental costs and potentially puts at risk law enforcement and the implementation of environmental policies.

To ensure that ecosystem services are evaluated from the initial damage assessments, a framework for deforestation cost evaluation has been developed, based on Habitat Equivalency Analysis (HEA), as first described by the National Oceanic and Atmospheric Administration (NOAA) and in line with the European Union Liability Directive.<sup>1,2</sup> The framework considers remediation costs as proxies for evaluating ecosystem services, and it enables the calculation of interim losses (i.e., the time elapsed between the loss of ecosystem services caused by deforestation and the return of these ecosystem services after damage recovery).

Four study areas of the Brazilian Atlantic rainforest biome were evaluated using the framework. All four study areas involved a forest structure at the intermediate stage of regeneration prior to the damage: two areas had been converted to pasture; one area had been converted to a *Eucalyptus spp.* Plantation; and one area had had its soil removed by mining activities, causing the damage to be considered perpetual.

The application of the framework resulted in an increase in environmental valuation by 38%–43% in pasture areas, 33% in the *Eucalyptus spp.* area, and 232% in the mined area, without considering land acquisitions. Ratios between adapted forest ecosystem services values described by Costanza et al. and framework values were 1.65–2.23 in pasture areas, 1.75 in *Eucalyptus spp.* areas, and 4.85 in mined areas.<sup>3</sup> The inclusion of land acquisitions in these calculations would push these ratios toward 1.

The results demonstrate the potential of the framework to inform the forensic science community, improving the ability to evaluate interim losses, perpetual damages, and propose better approximations of the total economic value of deforestation. Considering the extent and consequences of deforestation in Brazil, the proposed framework reveals the actual cost of environmental damages and the negative economic impacts of deforestation.

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#### Environmental Damage Cost, Ecosystem Services, Habitat Equivalency Analysis



### D36 A Fatal Injury Caused by a Scissor Jack in a Rear-End Collision

*Kurt D. Weiss, MS\*, Automotive Safety Research, Santa Barbara, CA 93111-2326*

**Learning Overview:** The goal of this presentation is to discuss a crash in which a seat belt-restrained occupant in a Sports Utility Vehicle (SUV) was fatally injured in rear-end collision. The physical evidence and the interior configuration of the vehicle will be detailed, and the mechanism of the fatal injury will be clearly explained.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the process of examining the physical and photographic evidence, and the vehicle interior configuration, that lead to the conclusion that vehicle occupants may be exposed to unwanted injury in foreseeable collisions.

Vehicle traffic had stopped for several minutes due to a previous traffic collision ahead. The three lane Interstate with a posted speed limit of 70mph had become a parking lot. Near the back of the line of cars, the operator of a long-haul tractor-trailer switched on his rig's four-way emergency lights. Stopped right behind his trailer was an SUV. Tragically, the driver of the SUV had no way to avoid the impending rear-end collision.

The SUV was struck by a small sedan, the driver of which claims to have fallen asleep. His speed control was set to 70mph–75mph. Contrary to his statement, he attempted to avoid the impact by steering left, evidenced by an approximately 31-foot-long angled tire friction mark. The right front area of the sedan struck the left rear area of the SUV in the offset rear-end collision. The maximum penetration to the SUV centerline was approximately 28in.

Upon impact, the sedan continued forward and entered the natural desert terrain of the center median, where it slowed to a rest. The SUV rotated clockwise and was pushed into the left rear corner of the trailer, evidenced by hinge hardware impressions to the SUV's left side. The frontal and left side curtain shield and torso airbags had deployed. The slack webbing confirmed the driver's seat belt was fastened at the time of collision. Vehicle rotation continued until the SUV's left rear corner struck the trailer's left side.

The SUV driver was extricated and flown by air ambulance to the local trauma center with a Glasgow coma scale of 3, and radiological findings of subdural and subarachnoid hemorrhages. A brain flow study showed the absence of circulation and death was pronounced. In the county coroner's opinion, the driver of the SUV died as a result of blunt force head trauma. The coroner's report noted a defect to the superior aspect of the occipital region of the head, at the superior point of the lambdoid suture. The report also noted multiple emanating linear skull fractures from this defect.

The SUV was examined, and it was discovered the rear damage profile involved the bumper reinforcement beam, lower edge of the liftgate, suspension components, and tire assemblies. Intrusion at the floor level compromised the convenience spare tire well located in the rear cargo area.



Collision forces overwhelmed the wing bolt and thrust the spare tire into the vehicle compartment. This fact was supported by finding the deformed wing bolt and a corresponding hole in the spare tire support.

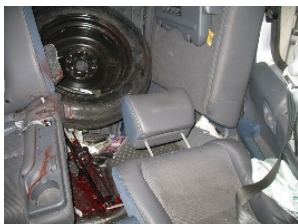


Additionally, numerous rubber transfers were observed to the front of the spare tire well.



An eye-witness stated they had moved the spare tire from the driver's head and neck area to open the airway. However, the head injury is inconsistent with impact solely by the spare tire.

Investigator photographs taken at the collision scene show the driver's seat back reclined.



However, it is unknown if the seat back was reclined to facilitate extrication or if the recline mechanism failed as a result of the collision. On the floor behind the driver's seat is the spare tire, and next to the seat back is a blood-covered scissor jack.

An exemplar vehicle was examined to evaluate the spare tire storage hardware. It was determined that the scissor jack was secured beneath the spare tire.



The rear seats of the SUV permit full reclining, and additional cargo space may be created by folding each rear seat to each side wall separately. In this case, the left rear seat assembly was stowed along the side wall, whereas the right rear assembly remained upright.



This rear seat configuration allowed a clear path for the scissor jack to be propelled directly into the head, killing the driver.

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### SUV, Scissor Jack, Blunt Force Head Trauma



### D37 Positional Asphyxia

David Pienkowski, PhD\*, University of Kentucky, Lexington, KY 40536-0298

**Learning Overview:** After attending this presentation, attendees will: (1) review the musculoskeletal mechanics of breathing, (2) examine known mechanisms of positional asphyxia, (3) review two cases involving different mechanisms of positional asphyxia, and (4) gain a broader perspective on the biomechanics of death not otherwise associated with penetrating injuries or other mechanisms that disrupt human ventilation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing new insights into death caused by positional asphyxia, specifically those attributable to accidental external ventilation restrictions.

Living organisms energized by oxidative phosphorylation of Adenosine Triphosphate (ATP) require oxygen (O<sub>2</sub>) for cellular (internal) respiration. O<sub>2</sub> is provided by circulating blood that exchanges cellular by-products (Carbon Dioxide (CO<sub>2</sub>)) with O<sub>2</sub>-rich air in the lungs. Breathing (external respiration) involves active cyclic muscle-force generated, O<sub>2</sub>-rich atmospheric air induction into the lungs followed by passive exhalation of CO<sub>2</sub>-rich gas. The lungs are incapable of self-movement and rely on volume changes of the upper chest cavity for CO<sub>2</sub> and O<sub>2</sub> transport. Contraction of the diaphragm and intercostal muscles pull the ribs superiorly and anteriorly, thereby increasing chest cavity volume. This expanding lung volume creates a negative internal pressure resulting in air inhalation. Exhalation is a passive process whereby these muscles relax, the ribs move inferiorly and posteriorly, the volume of the chest cavity decreases, and gas in the lungs is expelled. Sustenance of adequate cellular respiration requires removal of CO<sub>2</sub> and delivery of O<sub>2</sub>, which requires movement of a specific volume of air in the respiratory system. This volume must also include sufficient additional to compensate for the “dead air space” in the mouth and trachea. Air in this dead air space does not participate in effective exchange of CO<sub>2</sub> and O<sub>2</sub>.

Positional asphyxiation occurs when expansion or relaxation of the ribs cannot occur to a sufficient extent to provide adequate gas exchange. Positional asphyxia may occur with flexed or extended torsos such that adequate musculoskeletal chest movement is prohibited or when immovable external objects obstruct such movements. Positional asphyxia has occurred in industrial accidents, infants belted in some automotive child seat designs, and prisoner restraint. The following describes instances of positional asphyxia for which inhalation or exhalation was inhibited.

**Case 1:** An 18-wheel truck carrying coil sheet steel, estimated weight approximately 38,000 pounds, struck the rear of a second 18-wheel truck. Delta-V of this collision was approximately 35mph–40mph. Intrusion of the bullet tractor into the target trailer crushed the bullet tractor and limited space forward of the bullet tractor driver. Restraints securing the coiled steel cargo failed and, upon impact, this cargo translated forward and deformed the back of the bullet tractor cab. The back of the cab behind the driver pressed the driver’s seat forward into the area already compacted by impact of the bullet tractor with the target trailer. Although the driver of the bullet tractor sustained no rib fractures, the combined forward and rearward intrusion of the cab flexed his hips and knees toward his chest. This position, in conjunction with the driver’s surfeit of adipose tissue, restricted adequate ventilation of the driver. Eyewitness reports indicated that shortly after the collision, the driver was alert and urgently requested extrication. Unfortunately, the necessary equipment did not arrive in time. The victim expired on the scene *in situ*. Positional asphyxia occurred due to the inability to adequately inhale.

**Case 2:** A young man was reaching over the third-row seat of a Honda® Odyssey® minivan. Unable to reach the item of interest, he rose from the seat, turned 180°, placed his knees on the seat, leaned over the seat back, bending forward at the waist. The seat rotated toward the back bumper of the van, resulting in pinning the man upside down with the top of the back seat pressed against the inferior aspect of his ribs. The victim was unable to right himself and simultaneously rotate the seat to the normal position. The position of the seat against the ribs partially inhibited diaphragmatic relaxation and inferior rib movement. Adequate gas volume exhalation did not occur. First responders did not arrive in time, and the man expired on the scene due to positional asphyxia believed attributable to the inability to adequately exhale.

#### Asphyxiation, Accidental Death, Biomechanics of Breathing



### D38 Seat Belt Load Marks on Aged Restraint Systems

*Mike Markushewski, BS\*, ARCCA, Inc, Penns Park, PA 18943*

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**Learning Overview:** The goal of this presentation is to show that loading patterns on aged restraint systems are lighter as compared to new systems when exposed to the same crash forces.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by investigating the relative difference in restraint system loading patterns on new versus aged restraint system components. A difference in loading patterns is expected as a result of typical aging characteristics on polymer materials and their respective response to impact loading.

Polymer-coated components used on the typical seat belt system are the D-ring or B-pillar guide loop and the latch plate which inserts into the buckle. The D-ring provides a turning and load surface for the webbing as it exits the locking retractor and forms the upper anchor for the shoulder belt. The latch plate controls the confluence of webbing between the lap and shoulder belt. The primary purpose of the coating is to provide a low-friction surface for the polyester seat belt webbing to slide on. The polymer surface on the D-ring and latch plate permits the webbing to slide freely in response to normal occupant motion while driving in addition to providing proper coupling of the occupant to the vehicle during a crash.

A dynamic horizontal accelerator test program was conducted to evaluate documented older seat belt systems as compared to new replacement restraint systems in a controlled manner. In an effort to isolate the differences in loading patterns on the restraint systems, as many variables as possible are to be controlled. New seat belt systems and older seat belt systems are mounted on the horizontal accelerator platform using identical generic, but typical, restraint system geometries. Two surrogates are used and ballasted to a 50th percentile male or approximately 167 pounds.

The main purpose of this study is to attempt to show the difference in loading patterns on aged seat belt polymer components that have been exposed to the same crash forces compared to new seat belts. Physical properties of polymers typically will change as a result of exposure to heat, humidity, ultraviolet light, and sunlight through, in some part, the photo-oxidative degradation process. Leaching of plasticizers, if used, whose purpose is to maintain flexibility of polymer chains, also results in increased surface hardness as the material ages.

It is suggested that because the new polymer materials are softer, the load surfaces will be more prominent and heavier loading patterns will be visible on surfaces that have been exposed to crash forces, as compared to aged and therefore harder, seat belt components for a given acceleration and delta-V. Conversely, the presumption is that aged polymers tend to increase in surface hardness and therefore will display lighter load marks as compared to new seat belts for a given impact.

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#### Seat Belt, Load Marks, Polymers

### D39 Shared Anchor Seat Belt Buckle Self-Release

Kurt D. Weiss, MS\*, Automotive Safety Research, Santa Barbara, CA 93111-2326

**Learning Overview:** After attending this presentation, attendees will understand about a seat belt-restrained occupant in a Sports Utility Vehicle (SUV) who was ejected and fatally injured in a rollover collision. The physical evidence will be detailed, and the mechanism of inadvertent seat belt buckle release will be clearly explained.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how a shared anchor seat belt component design can be susceptible to inadvertent release.

Federal safety standards require that seat belt assembly hardware shall be designed to prevent attachment bolts and other parts from becoming disengaged from the vehicle while in service.<sup>1</sup> Failure of seat belts at or near the anchor points can render vehicle occupants unrestrained and particularly vulnerable to ejection. It is not uncommon for two adjacent seat belt assemblies to share an attachment bracket and anchor point. A previous study demonstrated how a design change of a shared anchor buckle component subjected to reasonably anticipated forces resulted in catastrophic failure and occupant injury in a rollover collision.<sup>2</sup> The current study highlights another shared anchor design that is susceptible to inadvertent release.

Four occupants of an SUV were northbound at approximately 65mph. A vehicle eastbound at approximately 26mph failed to stop at a stop sign and struck the SUV in the left rear corner. After the initial contact, both vehicles began to yaw counterclockwise. The SUV tipped on its right-side wheels and overturned on the passenger side, leading 4¾ times. By all accounts, the occupants of the SUV were wearing their type-2, lap and shoulder belts. Physical evidence, one in particular, a paint transfer to the seat belt webbing matching the vehicle's exterior color, validated claims of belt use for the second-row left occupant.



Despite this fact, the left rear occupant was ejected and sustained a fatal blunt force head trauma, including skull fractures, brain hemorrhages, and near-complete transection of the brain stem. Occupant ejection while wearing a seat belt is not a unique occurrence.<sup>3-5</sup>

Vehicle inspection revealed the second-row left buckle was a shared anchor component with the adjacent center buckle.



The left buckle is attached to a stiff metal bracket whereas the center buckle is attached by a webbing strap. When installed, the left buckle is slightly recessed while the center buckle lies atop the seat cushion.



In this case, the second-row left and center seats were both occupied at the time of collision.

Design considerations of seat belt buckles involve such things as comfort, convenient access, and performance. It was proposed that the second-row buckle design met some vehicle manufacturers' specified resistance to opening when probed with varying diameter balls (e.g., General Motors® 40mm, Nissan® 40mm, Ford® 38mm, and Toyota® 35mm ball). Yet, the preponderance of evidence indicated the second-row left buckle was most likely inadvertently released during the collision.

Closer examination revealed curious artifacts to the plastic cover of the left buckle.



Furthermore, the lower corner of the adjacent center buckle has a smaller diameter than the balls used in resistance to opening testing. Several new exemplar components were obtained and tested. The elongation of the attachment webbing of the center buckle under reasonably anticipated tensile forces was demonstrated. Alignment of the buckle housing allowed for its lower corner to interact with the adjacent left buckle. Therefore, it was correctly concluded that interaction of the adjacent buckles in use at the time of collision caused inadvertent buckle release that resulted in the ejection and fatal injury of the second-row left-seated occupant.

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### Shared Anchor, Inadvertent Buckle Release, Ejection Fatality



### D40 Seat Belt Analysis: Sometimes Plastic Transfer to a Webbing Indicates the Restraint Was NOT in Use During a Crash

David J. Porta, PhD\*, Bellarmine University, Louisville, KY 40205

**Learning Overview:** After attending this presentation, attendees will understand the typical and atypical evidence of restraint system loading during a motor vehicle crash. The role of pretensioner deployment will be demonstrated in a moderate frontal collision.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing important information for any reconstructionist desiring to determine the relationship between restraint use and injury production.

In a collision described as very severe by police investigators, the front of a 2006 domestic sedan struck the left side of a 2013 tractor with trailer as they met at an intersection. Both second-generation front airbags in the sedan deployed (first stage only). The 5' 9" tall, 180lbs, 46-year-old female driver of the sedan was noted by police and Emergency Medical Services (EMS) as having been properly three-point restrained, as was her passenger. She was transported to the hospital with several complaints, including a closed head injury. The driver was released the next day and eventually filed suit claiming a traumatic brain injury. The passenger suffered a shoulder injury that required considerably more medical treatment, but she did not participate in the lawsuit. As part of a thorough accident reconstruction, the crash data recorder was downloaded, and it indicated the sedan experienced a change in velocity of 25mph at impact. This is well below the severity of impact in the United States Department Of Transportation (DOT) New Car Assessment Program frontal crash test for this model vehicle (New Car Assessment Program (NCAP) Test 4931). The NCAP test involved a 50 percentile male Anthropomorphic Test Dummy (ATD) of similar stature to the female driver in this case. The sedan earned a maximum five-star rating indicating the chances of serious injury at 10% or less in a 35mph frontal crash into a barrier. Test videos indicate the head and torso of the properly restrained ATD rotated into the deploying airbag and did not contact the headliner, dashboard, or windshield. The data recorder further indicated the sedan driver seatbelt was unbuckled, but the front seat passenger belt was buckled. An investigation of the vehicle was undertaken in order to rule out restraint system failure. Upon inspection, the passenger restraint system showed typical abrasions to the cabin-facing surface of the D-ring and concomitant plastic transfer to the seatbelt webbing—clear evidence of restraint use during the collision. The driver seatbelt webbing was found cut and exhibited similar plastic transfer from the D-ring. Without further examination, one might assume the driver had been properly restrained. However, the webbing was locked at a relatively unspooled length. A driver side retractor-mounted pretensioner clearly fired while the belt was in the stowed position. As the belt was quickly pulled into the spool, it produced abrasions to the inside of the plastic-coated D-ring (the side facing the B pillar). There was very clear evidence of plastic transfer to the belt at a location that would correspond to it being retracted at the time of firing. No evidence or testimony explained why the belt was cut, but it is not uncommon for tow truck operators to cut and use seatbelts to secure a door or the steering wheel.

The driver claimed to suffer a head injury, and, at inspection of the vehicle, it was noted that there were abrasions to the visor and a fracture to the windshield. Both were reasonable locations for head contact by an unrestrained driver—NOT one who was properly restrained, of a size similar to NCAP ATDs, and in an accident that was approximately 30% less severe than NCAP tests.

It is unknown how many vehicles have crash data systems programmed to trigger a pretensioner to fire on an unbuckled seatbelt, but this presentation clearly demonstrates one such case and attendees will now know of this possibility, and the evidence for it, when performing an analysis of a restraint system.

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#### Seat Belt, Pretensioner, D-Ring



### D41 Void Analysis in the Inside of Arc Melting Using 3D X-Rays

*Young Jin Cho\**, National Forensic Service, Wonju-si, Gangwon-do 26460, SOUTH KOREA; *Dong Gyu Lee, MA*, National Forensic Service, Wonju-si 26460, SOUTH KOREA; *Sun Jae Kim, MA*, National Forensic Service, Wonju-si 26460, SOUTH KOREA; *Jong Teak Park, MA*, National Forensic Service, Wonju-si 26460, SOUTH KOREA; *Jae-mo Goh, PhD*, National Forensic Service, Wonju-si, Gangwon-do 26460, SOUTH KOREA; *Nam-Kyu Park, PhD*, National Forensic Service, Wonju, SOUTH KOREA

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**Learning Overview:** After attending this presentation, attendees will better understand the feasibility of using 3D X-rays as a tool for the Void analysis of molten marks.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing an effective tool for the Void analysis of molten marks.

The arcing damage to electrical copper wires is often found at a post-fire scene where the electrical installation was energized during the early development of the fire. This damage can be useful to the investigation as it can provide reliable data to establish a fire's area of origin.

The primary arc melting refers to the arc melting acted as a cause of a fire, and the secondary arc melting refers to the arc melting formed while the insulated covering of electric wires is combusted after a fire breaks out. Since being able to distinguish the primary arc melting from the secondary arc melting means that it is possible to judge clearly whether it is acted as a cause of a fire or not, it could be very assistive in investigating causes of a fire. However, a definite method to discriminate this does not exist yet.

Researchers have studied diverse methods in order to distinguish the primary arc melting from the secondary arc melting for decades in the past, and one of those methods is to analyze the void inside the arc melting. It is said that the primary arc melting can be distinguished from the secondary arc melting by analyzing the number of voids, the size of voids, the location of voids at the cross section etc. but since the results of each researcher are different, it is also difficult to apply to actual situations.

The method analyzing voids can be used for researches to distinguish the arc melting from the fire melting apart from discrimination of the primary arc melting from the secondary arc melting.

To examine Void in the inside of molten marks, metallurgical analysis can be used. However, this method takes too much time due to many stages (cutting, molding, grinding, polishing, and etching), and it is only capable of observing one cross section.

This study, using Void analysis among the functions of software enabling 3D X-rays, determines that it is possible to easily and quickly interpret the proportion of Void in the volume of molten marks, the distribution of Void, and the diameter of Void. In addition, this study also shows that using the function of Void analysis should be much more precise and efficient than the method of physical cutting.

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### **Void Analysis, 3D X-Ray, Molten Mark**





### D42 The Distinction Between Arc Melting and Fire Melting Using X-Rays

*Jae-mo Goh, PhD\*, National Forensic Service, Wonju-si, Gangwon-do 26460, SOUTH KOREA; Young Jin Cho, National Forensic Service, Wonju-si, Gangwon-do 26460, SOUTH KOREA; Dong Gyu Lee, MA, National Forensic Service, Wonju-si 26460, SOUTH KOREA; Sun Jae Kim, MA, National Forensic Service, Wonju-si 26460, SOUTH KOREA; Jong Teak Park, MA, National Forensic Service, Wonju-si 26460, SOUTH KOREA; Nam-Kyu Park, PhD, National Forensic Service, Wonju, SOUTH KOREA*

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**Learning Overview:** After attending this presentation, attendees will better understand the feasibility of using X-rays as a tool for the distinction between arc melting and fire melting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing an effective tool for the distinction between arc melting and fire melting.

Molten marks found on copper wires at the scene of the fire can be largely divided into primary arc melting, secondary arc melting, and fire melting. For decades, many studies have been conducted to make a distinction between primary arc melting and secondary arc melting, but it is only possible to partially differentiate them under limited conditions.

The method of using Void in the inside of arc melting, among the methods proposed to differentiate between primary arc melting and secondary arc melting, mostly shows conflicting results. The results of preceding research and literature related to Void indicate that, although it is difficult to differentiate between primary arc melting and secondary arc melting, there are various types of Void in the inside of arc melting and that there are a much smaller number of Void in the inside of fire melting than that of arc melting.

Arc melting is characterized by the external visual indicators of the following: sharp demarcation between damaged and undamaged area, round shape of artifact, resolidification waves, etc. In contrast to melting caused by an arc, fire melting is characterized by the external visual indicators of the following: visible effects of gravity on the artifact, extended area of damage without a sharp demarcation from undamaged material, gradual necking of the conductor, etc.

The Void analysis method can be used to differentiate between arc melting and fire melting when external visual indicators are not sufficient or are disputed.

In this study, the distribution of Void in the inside of molten marks was examined using 2D and 3D X-rays as well as appearance tests to devise a method of differentiating between arc melting and fire melting, and, as a result, it was found in some cases that using X-rays to check the distribution of Void could lead to a clear distinction. Other than non-destructive inspection using X-rays, it is also possible to observe Void in the inside of molten marks with a microscope, but it takes time due to many stages (cutting, molding, grinding, polishing, and etching), and it allows an observation only on one cross section, which implies that using X-rays is much more precise and efficient in checking the presence of Void.

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#### Arc Melting, Fire Melting, X-Rays



## E1 The First Cut Is the Deepest

Tyrish Y. Page, MA\*, *Sharing Hope SC, Charleston, SC 29405*; Jeff Thomas, MBA\*, *Sharing Hope SC, Charleston, SC 29405*; Angelina I. Phillips, MD\*, *MUSC, Charleston, SC 29425*

**Learning Overview:** After attending this presentation, attendees will understand that tissue procurement can preclude forensic autopsy without repercussions to the death investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the need to establish protocols between coroner/medical examiners and organ procurement organizations for pre- and post-autopsy tissue procurements.

Organ and Tissue Procurement Organizations' (O/TPOs) optimal goal is to maximize the "gift of life." The "gift of life" is offering hope and changing lives through organ, eye, and tissue donation. Tissue procurement must begin within 24 hours of the patient reaching asystole. This process may collide with the death investigation being conducted by the Medical Examiner and/or Coroner (ME/C), especially if an autopsy is necessary. The result has become a struggle about access to the body that pitted the transplant community's promise of "the gift of life" against the medical examiner's mandate to determine the cause of death in forensic case.<sup>1</sup> In 2017, 182 potential tissue cases were declined by coroners in South Carolina. These cases included donors who were eligible for either pre- or post-autopsy tissue procurement. Some ME/Cs deny procurement of organs and/or tissues for transplantation due to concerns of not being able to fulfill their legal mandate to determine the cause and manner of death, and to ensure that appropriate evidence is collected. With appropriate communication and cooperation between ME/Cs and O/TPOs, this should not be the case in the vast majority of situations.<sup>2</sup>

A referral for a 21-month-old female was called into Sharing Hope SC post-cardiac arrest from choking on a chewy candy, and the patient rapidly declined to death after admission. Organ procurement was unable to proceed due to the rapid decline, but the tissue procurement team immediately contacted the coroner for possible approval to procure. The coroner spoke with the family and medical examiner prior to giving authorization to procure heart valves. The coroner declined to have an autopsy but requested photos and a cardiac pathology report upon completion. During tissue procurement, the technician noted pulmonary edema and the heart was recovered for tissue processing. A detailed report provided by the processor's pathology laboratory examined the decedent's heart, myocardial thickness, coronary arteries, and valves and noted everything to be unremarkable. Several months later, the cause of death was amended due to the relaunching of the death investigation. The pre-autopsy photos and tissue procurement reports were momentous to the investigative team. The body was exhumed, an autopsy was completed, and the cause was changed to homicide.

Examining the heart during autopsy can be essential to the medical examiner when determining cause of death. Many O/TPOs and C/MEs have created and collaborated on methods to examine the heart while not disrupting the procurement of the allograft for transplantation. The result is that the medical examiner is able to determine and document the cause of sudden death, and heart valves that would previously have been denied are made available for transplantation.<sup>3</sup> Size-critical heart valves or tissue grafts are frequently requested by surgeons for patients that suffer from congenital defects of the pulmonary or aortic valve. Heart valves, especially pediatric heart valves, are in critical need. Nonetheless, the availability of homografts, especially in the smallest ranges, varies due to the limited donor pool.<sup>4</sup> This case study will highlight the need for pre-autopsy tissue, establish a protocol that MEs can visually examine the heart valve within a sterile environment and preserve evidence during pre-autopsy tissue procurements, and address ways the C/ME and O/TPO can work together to maximize the "gift of life" as well as be the voice for those who can no longer speak.

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### Tissue Procurement, Coroner/Medical Examiner, Modified Cardioectomy



### E2 Which Came First, the Chicken or the Egg? A Key to Shed Light on a “Road Murder”

*Giuseppe Bertozzi, MD\*, Department of Forensic Pathology, Foggia 71121, ITALY; Stefania De Simone, MD, Department of Forensic Pathology, Foggia, Foggia 71122, ITALY; Michela Ferrara, MD, Department of Forensic Pathology, Foggia 71122, ITALY; Lorenzo Spagnolo, MD, Department of Forensic Pathology, Foggia, Foggia 71122, ITALY; Giuseppe Davide Albano, MD, Foggia 71121, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY; Francesca Maglietta, MD, Department of Forensic Pathology, Foggia 71122, ITALY; Monica Salerno, MD, PhD, Department of Forensic Pathology, Foggia 71121, ITALY*

**Learning Overview:** The goal of this presentation is to underline the discrepancies between antemortem and postmortem instrumental diagnosis, confirming autoptic examination as the gold standard for forensic diagnosis. This is true even in cases of traumatic death, such as a traffic accident, in which radiology showed its indubitable function.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that a whiplash injury in an elderly patient with age-related vasculopathy can lead to the onset of an ischemic process, causing death. Even as a rare pathophysiological mechanism, this has to be considered when approaching cases as the one settled previously. Moreover, the forensic pathologist has to know the in-depth anatomy in order to adapt the dissection techniques to each specific case, even borrowing them from other surgical specialties. This need for continuous updating and a critical approach to the autopsy cannot help but be assisted by the new pre-autoptic technologies, always remembering that the macro- and microscopic studies remain irreplaceable.

Whiplash is a traumatic event affecting the cervical spine. In most cases, it arises following a sharp movement of the head that exceeds the physiological limits of joint excursion. Many studies document the presence of ischemic accidents following the compression of the vertebro-basal system arteries after whiplash in predisposed patients.

This case dealt with a 79-year-old man driving his own car who was involved in a frontal impact crash after he was hit by another car. He was transported to the emergency department, where the driver arrived comatose (GCS 3). Immediately, the patient underwent a brain Computed Tomography (CT) scan and angio-CT scan, directed, in particular, to study the epi-aortic vessels. The CT scan showed a wide ischemia of cortical and subcortical areas, particularly affecting the parietal and occipital encephalic lobes and cerebellum. The angio-CT scan revealed the complete occlusion of the lumen of both vertebral arteries at the level of the third cervical vertebra. The man died approximately four days after his admittance to the hospital.

The external examination of the body was not remarkable for any signs of trauma. The question was if the ischemia or the car accident came first.

Before performing the autopsy, a CT scan of the skull and neck was conducted. The autopsy was performed six days later, with a particular dissection protocol borrowed from the neurosurgery. The examination was focused on the vertebral artery segments between the third and fourth cervical vertebrae. During the dissection, an open-book cervical vertebral fracture was identified, leading to the complete obstruction of vertebral arteries, bilaterally. The histological examination of these vascular segments revealed the presence of a hemorrhagic infarction surrounding the adventitial layer, compatible with vitality at the time of its genesis.

Whiplash was the cause of death!

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#### Whiplash, Traffic Accident, Vertebral Arteries

### E3 Bilateral Internal Carotid Arteries Dissection in Motorcycle Accidents: A Case Report and Review of the Literature

Caterina Petetta, MD\*, Department of Public Health and Pediatrics, Torino 10126, ITALY; Niccolò D. Melloni, MD\*, Department of Public Health and Pediatrics, Turin 10126, ITALY; Lucia Tattoli, PhD, Struttura Complessa di Medicina Legale, Torino, Turin 10126, ITALY; Davide Santovito, MD, Department of Public Health and Pediatric Sciences, Turin 10126, ITALY; Giancarlo Di Vella, MD, PhD\*, University of Torino, Department Public Health Sciences, Torino 10126, ITALY

**Learning Overview:** After attending this presentation, attendees will understand the importance of suspecting and evaluating cervical and intracranial vessel injuries in all patients involved in motorcycle accidents. Bilateral Internal Carotid Artery Dissection (ICAD) due to blunt head and neck trauma has an estimated incidence of 1%–1.7%.<sup>1</sup> It may also occur in motorcycle collisions, resulting in a life-threatening event if not promptly diagnosed and treated. Mortality rates for this injury are reported from 20% to 40%, while permanent neurologic deficits range from 40% to 80%.<sup>2</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that while bilateral ICAD in motorcycle accidents is very rare, timely diagnosis and treatment may prevent death or permanent neurological deficit. Early recognition is difficult and often delayed due to commonly associated severe multisystem injuries and the latent interval before the onset of clinical symptoms.

A case of a 44-year-old man involved in a high-speed motorcycle accident (frontal collision with a car) who sustained severe injuries, including bilateral ICAD, was reported. He was admitted to the emergency department unconscious, with severely altered vital sign parameters and bone and visceral injuries to the thorax and the pelvis. An initial brain Computerized Tomography (CT) scan was unremarkable. Because of his prolonged altered state of consciousness, a second cerebral CT scan and CT angiogram were performed. These studies revealed bilateral ICAD with thrombosis, treated non-operatively with fibrinolytic and anticoagulants, five days after the accident. The patient survived and regained consciousness. After multiple orthopedic and urological surgeries, he was discharged to initiate neuromotor rehabilitation and language therapy for the following two months. The bilateral ICAD has caused cerebral ischemic damage, resulting in upper and lower right limb hemiparesis and bradyphasia.

The literature review was conducted by the PubMed database with the goal of highlighting the importance of the traumatic mechanism of ICAD, risk factors, and its consequences for patient care and management. Papers regarding typical motorcycle accident injuries ( $n=24$  potentially relevant results), and bilateral ICAD due to blunt trauma ( $n=18$ ) were searched. The cross-search revealed motorcyclists are frequently exposed to direct traumatic injuries due to impact with: (1) rigid structures of the motorcycle (tank, handlebars, etc.); (2) collision vehicle; or (3) road pavement or other fixed obstacles on the scene at the time of projection to the ground. Typically, motorcyclists sustain abrasions, extremity fractures or avulsions, head and neck trauma frequently with skull base fractures, encephalic and spinal cord lesions, crushing thoraco-abdominal injuries, and perigenital and genital injuries. They may also suffer blunt force indirect injuries caused by rapid and sudden deceleration occurring at the moment of collision. This often causes traction and tearing of the organs held in place by vascular structures and/or ligaments. Among case reports of bilateral ICAD due to blunt trauma, none of these described this peculiar lesion in motorcycle victims, while the majority of cases occurred in horseback-riding, snowboard, or automotive vehicular collisions.

In the case reported, from a medical-legal standpoint, the man suffered both typical injuries due to direct trauma (such as abrasions, head, neck and thoraco-abdominal trauma, multiple pelvic fractures, and perigenital and genital injuries) and indirect trauma (such as bilateral ICAD).

Results from the literature review indicate that the early diagnosis of traumatic ICAD is important in order to prevent further complications. It should always be suspected in cases of motorcycle accidents as well as in blunt neck trauma with hyperextension or cervical spine fractures, especially in the presence of neurologic deficits not explained by head trauma.

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#### Bilateral Carotid Artery Dissection, Blunt Force Trauma, Motorcycle Accident

## E4 Gone in 60 Seconds: A Fatal Case of Asphyxiation in a Confined Working Space

Giuseppe Davide Albano, MD, Foggia 71121, ITALY; Giuseppe Bertozzi, MD\*, Department of Forensic Pathology, Foggia 71121, ITALY; Mauro A. Ciavarella, University of Foggia, Forensic Department, Foggia 71121, ITALY; Stefania De Simone, MD, Department of Forensic Pathology, Foggia, Foggia 71122, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY; Irene Riezzo, MD, PhD, University of Foggia, Osp D'Avanzo, Foggia 71100, ITALY; Francesca Maglietta, MD\*, Department of Forensic Pathology, Foggia 71122, ITALY

**Learning Overview:** The goal of this presentation is to provide useful evidence regarding asphyxiation findings in confined space deaths in cases which, according to external inspection in a death scene, a Carbon Dioxide (CO<sub>2</sub>) poisoning could be expected.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by establishing CO<sub>2</sub> poisoning cases when asphyxiation occurs in a confined space. When there are no specific CO<sub>2</sub> intoxication findings, the death scene and external investigation, gross anatomy, histology, and immunohistochemistry, as well as toxicological findings, are crucial to assessing asphyxiation features and to exclude other death causes.

CO<sub>2</sub>, a normal component of the atmosphere (0.02%-0.03%), a colorless and odorless gas, is physiologically produced by cellular metabolism and is widely used as an inert gas in fire extinguishers.<sup>1</sup> The toxicity of the CO<sub>2</sub> results from the effects to the respiratory center and from the replacement of oxygen. In low concentrations, it stimulates the respiratory center, and in high concentrations, it leads to respiratory depression and apnoea.<sup>2,3</sup> This data confirms how dealing with CO<sub>2</sub> is a great hazard. However, for forensic pathologists, the proof of CO<sub>2</sub> poisoning is still a great challenge.<sup>4</sup>

The presented case regards a 31-year-old man who was working as a fire extinguisher system maintenance person in the basement of a temporarily closed bank. There was only one main basement entrance. At that time, four other technicians were present. The anti-fire system was composed of several gas containers and many pipelines containing the inert CO<sub>2</sub> gas that was supposed to be released in case of fire and after the building evacuated. During maintenance activities, a massive gas leak from a container occurred. Four of the five men succeeded in escaping. The 31-year-old man was not able to get out and perished. Firefighters arrived and found his body without signs of life.

The forensic pathologist arrived and noted the position of the cadaver was a few meters from the gas container. The external examination revealed the presence of conjunctival petechiae, neck and face hypostasis localization, and no signs of injury. The autopsy, performed 24 hours after death, reported diffused brain and lungs edema, sub-pleural petechiae, and epicardial and myocardial petechiae. All vessels of the internal organs appeared to be congested. Histological investigation, with Hematoxylin and Eosin (H&E) stain, confirmed the gross anatomy findings. An increased number of alveolar macrophages were observed in the lungs as a consequence of protracted oxygen deficiency. Lung samples were processed for immunohistochemistry by using CD 68 and Hypoxia Induced Factor 1- $\alpha$  (HIF1- $\alpha$ ) antibodies. Positive reaction with macrophage marker CD 68 as well as HIF1- $\alpha$  was observed. Spectrophotometry analysis was performed in order to exclude high carboxyhemoglobin levels in periphery blood. Drug and alcohol concentrations in the periphery blood were not significant. Death scene information, and macroscopic, microscopic, and toxicological findings determined asphyxiation in a confined space as the cause of death.

Asphyxia in a confined space may be a consequence of oxygen exclusion because of depletion and replacement of another gas or as a result of chemical interference with its uptake and utilization.<sup>5</sup> In this case, a massive CO<sub>2</sub> leak from the fire extinguisher system occurred, and the basement was rapidly filled with CO<sub>2</sub>, which is denser than O<sub>2</sub> (1.98kg/m<sup>3</sup> vs 1.29kg/m<sup>3</sup>), leading to an oxygen exclusion. CO<sub>2</sub> intoxication is a diagnosis based on the scene investigation and circumstances surrounding a death with the exclusion of other causes of death.<sup>6</sup> There are no pathognomic autopsy findings and blood analysis for CO<sub>2</sub> content has no diagnostic significance, as CO<sub>2</sub> rapidly accumulates after death.<sup>7</sup>

In this case, the death scene investigation, autopsy, histological, immunohistochemical, and toxicological analysis were crucial to provide essential information in order to assess CO<sub>2</sub> intoxication. In such cases, CD 68 and HIF1- $\alpha$  antibodies use is essential in order to establish an oxygen deficiency and suggest asphyxiation death.

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### Confined Space, Asphyxiation, CO<sub>2</sub> Intoxication

## E5 Fatal Alveolar Capillary Dysplasia (ACD) in Two Siblings: A Rare Heritable Form of Persistent Pulmonary Hypertension in Neonates

Caterina Petetta, MD\*, Department of Public Health and Pediatrics, Torino, Italy 10126, ITALY; Giovanni Botta, MD, Department of Pathology, OIRM Sant'anna, Torino 10126, ITALY; Andrea Zonta, MD, Medical Genetics, Molinette Hospital, Torino, Italy 10126, ITALY; Eleonora Di Gregorio, PhD, Medical Genet. Città della Salute e della Scienza, Torino 10126, ITALY; Giancarlo Di Vella, MD, PhD\*, University of Torino, Department of Public Health Sciences, Torino 10126, ITALY

**Learning Overview:** After attending this presentation, attendees will understand the necessary elements for the diagnosis of ACD, a fatal lung disorder in premature or full-term infants. ACD is an uncommon interstitial lung disease causing Persistent Pulmonary Hypertension in Neonates (PPHN). Without responding to therapy, it is characterized with a mortality rate that approaches 100%.<sup>1</sup> The diagnosis of ACD is difficult because the etiopathogenesis of ACD and associated pulmonary hypertension remain to be fully explained.<sup>1</sup> Furthermore, an autopsy is not always performed after an infant's death, and ACD can be associated with other congenital system anomalies that are often considered to be the primary cause of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting that ACD must be suspected and immediately investigated in all infants with irreversible persistent fetal circulation. The literature review, conducted by the PubMed database, yielded 30 papers about ACD. It is described as a cyanogenic disorder characterized by failed postnatal decrease of the vascular pulmonary resistance, associated with the typical intrauterine right-to-left shunt. The disorder affects primarily the alveolar components leading to respiratory insufficiency early in life.<sup>2</sup>

Most ACD cases are *de novo* events but approximately 10% are heritable forms possibly involving siblings. 16q24.1 deletion and FOXF1 inactivating mutations have been identified as responsible in 40% of cases.<sup>1</sup> Currently, a definitive diagnosis depends on histological lung features found at autopsy or antemortem lung biopsy: (1) immature lobular development; (2) decreased number of pulmonary capillaries, dilated and located away from the alveolar epithelium; (3) thickened alveolar septa; (4) lymphangiectasis in interlobular septa; and (5) sometimes misalignment of pulmonary veins adjacent to pulmonary arteries.<sup>1</sup> In the case reported, two siblings born two years apart died after a severe and irreversible pulmonary hypertension with hypoxemia developed a few hours after their birth.

A 37-week gestational age (2,900g of weight, 9/9 Apgar score) male infant was born to a primipara via induced vaginal childbirth in view of polyhydramnios. The infant's respiratory status declined within the first 12 hours of life. Despite the immediate mechanical ventilation and inhaled nitric oxide, the neonate died 96 hours after birth because of irreversible respiratory failure. At autopsy, the histological examination of lung tissue showed immature and dilated alveolar capillaries distant from the alveoli through thickened septa, associated with lymphangiectasis. A 16p13.3 deletion was found through array Comparative Genomic Hybridization (CGH) analysis.

During the second pregnancy, the fetus shared the same 16p13.3 deletion through array CGH analysis on fetal DNA. Colon-sigma and rectum dilatation were detected by echocardiography at 17 weeks of gestation. A male infant was born by vaginal delivery at 38 weeks gestation (3,040g of weight, 6/9 Apgar score), but he developed serious pulmonary hypertension a few hours after birth. After detecting anorectal atresia, a right hemicolectomy was performed. Despite the regular clinical course, a respiratory failure occurred, not responding to any therapy until the infant's death 25 hours after birth. Autopsy and histological analysis showed underdeveloped acini and dysplastic and sparse capillaries within the thick interstitium in the lungs. In both cases, analysis of the lung tissues showed characteristic features of ACD as the cause of death. The recurrence of ACD and genetic tests (16p13.3 deletion) suggested an autosomal recessive or X-linked mode of inheritance.

Histological and genetic examinations have a key role in the diagnosis of ACD, especially in the prenatal period. An accurate assessment is fundamental for the management of PPHN because of the high rate of mortality. If death occurs, autopsy is recommended to verify system malformations. This approach may help to define the correct cause of death when related to ACD.

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### Alveolar Capillary Dysplasia, Neonates, Persistent Pulmonary Hypertension



### E6 A Young Mummy

*Giuliana D'anna, University of Bari-Section of Legal Medicine, Bari 70124, ITALY; Ilaria Santoiemma\*, Bari 70124, ITALY; Francesco Introna, MD, Dim Sezione Di Medicina Legale, Bari 70124, ITALY; Valeria Santoro, PhD, P.zza Giulio Cesare n.11, Bari 70124, ITALY*

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**Learning Overview:** After attending this presentation, attendees will be informed regarding a rare case of mummification that occurred in an unusual time interval.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that exceptions may exist in determining time since death in a complete mummified body.

A body in advanced stage decomposition is a challenge for forensic pathologists. Personal identification, as well as manner and time since death, require a specific skill, but sometimes they are not enough to explain all of the cadaveric phenomena.

It is known that mummification occurs in response to dryness of the environment, good ventilation, and high temperature. Due to the evaporation of water in the remains, the body becomes dehydrated or desiccated and shrivels. The natural appearances and features of the body are preserved indefinitely. Mummification is usually described after several weeks, and it is normally completed, on average, in a year.

In this case, a body in an advanced state of mummification was found during the summer season in an abandoned ground in southern Italy. The cranium was almost completely skeletonized, and the remaining part of the body was predominantly mummified. No signs of postmortem animal activity were found. No elements that allowed identification were found. The autopsy and the Computed Tomography (CT) scan excluded traumatic lesions, but the preservation of the organs' tissues, due to mummification, allowed histopathological analysis that concluded for a natural cause of death.

Police investigation revealed that 17 days before the discovery of the corpse, a missing Senegalese male was admitted to the emergency room after an accidental fall. During the admission, a CT scan was performed, but the subject left the hospital before completing all analysis, then he disappeared.

Odontological comparison was made between antemortem X-rays in the emergency room and skull X-rays performed at the Institute of Legal Medicine to compare the frontal sinus and odontological data. This provided a positive match between the mummified body and the missing Senegalese male.

Personal identification of the mummified body allowed the precise definition of the postmortem interval. The victim was in the emergency room 17 days before the discovery of the body, so one can conclude that the time of death was no longer than 17 days before the first inspection.

This case is interesting because of the atypical precocious mummification that occurred in a very short period of time (just two weeks) and for the procedure described by this study to obtain identification.

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#### **Mummification, Time Since Death, Personal Identification**



## E7 Mortality Among Hospitalized Injured Older Adults (HIOAs) for a Major Trauma: A Retrospective Autopsy Analysis (2000–2017)

Francesco Amico, MD, *Medicina Legale, Catania 95123, ITALY*; Giuseppe Davide Albano, MD, *Foggia 71121, ITALY*; Aldo Liberto, MD, *University of Catania, Catania 95123, ITALY*; Federico Patanè, MD, *Catania 95123, ITALY*; Dario Condorelli, *University of Catania, Catania 95100, ITALY*; Martina Fichera, MD, *University of Catania, Catania 95123, ITALY*; Diego Geraci, PhD, *Catania 95100, ITALY*; Monica Salerno, MD, PhD\*, *Department of Forensic Pathology, Foggia 71121, ITALY*

**Learning Overview:** Aging, injury, and hospitalization are a challenging triad for health care providers. In medical malpractice claims regarding HIOAs deaths, an Injury Severity Score (ISS) could be a very reliable evaluation parameter for forensic pathologists in order to establish health care workers' liability.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing useful and reliable data regarding HIOA deaths. Moreover, in such cases, when a malpractice claim occurs, death is a result of older adults' frailty, the trauma itself, as well as pre-existing medical conditions, leading to exclude health care workers' liability. This information needs to be taken into consideration in lawsuits regarding HIOA deaths.

Frailty is a leading feature in older adults and is characterized by physiological changes leading to an increased vulnerability to external stressors. A greater degree of frailty is associated with higher mortality risk and other adverse outcomes.<sup>1</sup> Trauma affects more than 9% of the United States population every year, and it is an important public health concern. In the United States in 2004, more than 2.9 million older adults were treated for traumatic injuries in hospital emergency departments.<sup>2</sup> Several studies suggested that older adults are at greater risk of fatal injury than their younger counterparts.<sup>3,4</sup> Furthermore, reliable data on fatal trauma is necessary for planning current and future trauma care in Europe.

This study reviewed all major trauma death autopsy databases regarding motor-vehicle crashes, cyclist investments, pedestrian casualties, falls from heights, accidental falls, aggressions, and accidents in the domestic and working place and was performed at the Department of Forensic Pathology of the University of Foggia from 2000 until 2017. Charts of all medical and forensic autopsies performed in the study were hand searched. Only cases with hospitalization previous to the death in patients older than 5 years of age were selected. The ISS is a method for numerically describing the overall severity of injury. It can be applied to those who have sustained injury to more than one area of the body as well as to those with isolated injuries. An individual's ISS is determined by rating each injury with the Abbreviated Injury Scale (AIS), then adding together the squares of the highest AIS rating for each of the three most severely injured areas. The ISS correlates substantially better with mortality than does the AIS rating for the most severe injury.<sup>5,6</sup> All cases with ISS greater than 15 were selected in this study. Demographic data, injury mechanism, pattern and severity, physiological signs, pre-existing medical conditions, and length of stay after trauma were obtained from pre-hospital trip charts, clinical charts, and forensic and medical autopsy records. In all malpractice claims, three different medical examiner offices, blinded to each other's opinions, evaluated the relationship between health care professionals' behavior and death.

Fifty-five autopsies were selected (35 M; 20 F; mean age 73, DS 5.4). Traumatic brain injury was the cause of death in 16 cases (29%), followed by pneumonia in 8 cases (15%), and Multiorgan Distress Syndrome, pulmonary embolism, and fat embolism (all in 6 cases, 12%). The median ISS was 21. The mean length of hospitalization was 27.78 days (median 15; I Q 6.5; III Q 29.5). In 43 cases (78%) pre-existing medical conditions were present. A negative correlation between time of survival after trauma and both number of pre-existing medical conditions and severity of trauma was observed.

In the case of HIOAs, undertriage remains a serious problem.<sup>7,8</sup> Treatment at a trauma center has been shown to be associated with a 25% lower mortality. In this study, in 32 of 55 cases (58%) hospitalization took place in a trauma center but in both the United States and Italy, there are no geriatric trauma centers.

In all cases, a correlation between health care professionals' behavior and death was excluded.

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### HIOA Deaths, Malpractice, ISS

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### E8 Funny Games: Homicide Among Neighbors

*Francesco Sessa, MS\**, Department of Forensic Pathology, University of FOGGIA, Foggia 71100, ITALY; *Michela Ferrara, MD*, Department of Forensic Pathology, Foggia 71122, ITALY; *Francesca Maglietta, MD\**, Department of Forensic Pathology, Foggia 71122, ITALY; *Giuseppe Bertozzi, MD\**, Department of Forensic Pathology, Foggia 71121, ITALY; *Irene Riezzo, MD, PhD*, University of Foggia, Osp D'Avanzo, Foggia 71100, ITALY; *Marcello Rendine, DBA*, Department of Forensic Pathology, Foggia 71100, ITALY; *Pietrantonio Ricci, MD, PhD*, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY; *Natascha Pascale, MD*, Foggia 71100, ITALY

**Learning Overview:** The goal of this presentation is to show how a multidisciplinary approach to the crime scene is instrumental in the study of complicated inspections to detect all traces of the crimes and to direct investigations in the correct manner.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how a complete forensic team could provide important support to investigative activities by providing benefits to law enforcement regarding the performance of forensic K-9 units and their optimized combination with forensic operators, revealing their capability in detection, identification, and location of biological traces.

This case dealt with a 55-year-old man who was killed in front of a building near his unauthorized cottage. The forensic pathologist team was alerted by the local prosecutor to a case of gun homicide. At the crime scene, the man was found lying on the sidewalk, supine, with his thorax in a large pool of blood. In front of the entrance door to the building were two steps. Near these steps, at a distance approximately 370cm from the corpse, and on the sidewalk, was a cartridge case. On the other side of the steps, at a distance of approximately 390cm from the corpse, another casing was found. The access door of the house appeared open, with a broken lower panel. Along the wall of this door was a window that had one of the two glass panes broken.

The external examination revealed, in correspondence with the fourth left intercostal space, the presence of a linear wound with finely jagged margins and extroflexes (exit hole). At the left upper abdominal quadrant, immediately below the costal arch, was a round-shaped wound with jagged and introflected margins (entrance hole). The left forearm presented with two wounds: one in correspondence to the upper third of the dorsal face, which was round shaped with jagged and introflected margins (entrance hole); the other in correspondence to the upper third of the fly face that was oval shaped with jagged and extroflexed margins (exit hole). In the right dorsal region was a roundish wound, with jagged and introflected margins (entrance hole). At the autopsy, an ogive was found in correspondence with the adipose tissue of the right gluteal region. The death was attributed to a cardiac and vascular lesion with cardiac tamponade. Such a wound is produced by a single-munition gun. The autopsy also identified other internal injuries produced by a gun.

During the interrogation, the suspect declared he was attacked first by the victim, who had used a golf club to break the windows of the house and the car before striking the suspect. The golf club was not found near the houses of the suspect and victim, so the prosecutor asked for a new inspection of the surrounding land because witnesses saw the victim's family hide the body. This inspection was effectuated with the help of the Forensic K-9 Unit and dogs trained to detect and locate any biological trace, such as blood.

The golf club was found buried in a field in front of the victim's house and was compared to the suspect's lesions, which were compatible with the aforesaid blunt body. Comparative genetic tests were conducted, comparing the blood DNA profile found on the club with the suspect profile obtained by buccal swabs. After genetic analysis, the profile matched, confirming the history told to the judge. In light of these findings, the judge decided to close the case as a legitimate (self) defense.

At the end of the police investigation, the suspect was declared innocent and returned to his home, near the house of the victim. After a few days, his house was set on fire. The police arson investigation is in progress.

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#### Forensic K-9 Units, Crime Scene Investigation, Genetics Analysis



### E9 A Mafia Ritual of Hiding Murders: Sheep in Boots!

*Francesco Sessa, MS\*, Department of Forensic Pathology, University of FOGGIA, Foggia 71100, ITALY; Elena Varotto\*, Casa di Cura “Santa Lucia” Centro Polidiagnostico, Siracusa 96100, ITALY; Laura Landini, MS, Laboratory of Archeozoology, Department of Civiliz, Pisa, ITALY; Francesca Maglietta, MD\*, Department of Forensic Pathology, Foggia 71122, ITALY; Santina Cantatore, Foggia 71100, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY*

**Learning Overview:** After attending this presentation, attendees will understand the modus operandi of an emerging Italian Mafia called Mafia of Gargano. A distinctive characteristic of the murders by this Mafia is the ritual of concealing the murder. Thanks to the karst conformation of this area, criminals frequently hide corpses in the natural ravines.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing scientific data on the multidisciplinary forensic approach used to support investigations, increase the gathered information, and help in the identification process of unknown human skeletal remains.

The Mafia is a highly organized Italian criminal organization that began in Sicily at the beginning of the 19th century. There are four Italian Mafia organizations, namely “Cosa Nostra” (Our Thing or Our Affair) in Sicily, “Camorra” in Campania, “Sacra Corona Unita” (SCU, United Holy Crown) in Puglia, and “Ndrangheta” in Calabria. In the Apulian territory (a region of Italy) there are several subsidiary independent criminal groups that modeled their organizations on that of the Camorra.

One of these is named “Mafia of Gargano” (Gargano identifies an area located in northern Puglia, also known as “The Spur of Italy”). This criminal association began its illegal activities in the 1990s with a series of violent killings committed between 1990 to the present.

In the first years of activity, this outlaw movement was underestimated by the local judges. In fact, they believed that these crimes were committed among rural families for cattle thefts. This organization became very important in the Apulian criminal scenario, of significant interest for several outlaw activities, such as drugs and arms trafficking. To date, the “Mafia of Gargano” has been responsible for approximately 198 murders, 40 of which are classified as “Lupara Bianca” murders. “Lupara” is the Sicilian word for shotgun, a firearm traditionally associated with mafia-type organizations. The term “Bianca” (white) symbolizes the absence of blood because bodies and traces of murders are concealed; for example, burying the corpses in the countryside, in places they would be difficult to find. This Mafia ritual is highly facilitated by the natural conformation of the Gargano promontory. Indeed, the presence of numerous karst ravines gives this territory a unique conformation, useful for hiding drugs, weapons, and corpses. Hiding corpses in the isolated areas of grottoes, valleys, and ravines that are hundreds of meters underground and far from the urban area allows for the body to be hidden, and the criminals can evade prosecution.

During military operations in the ravines located in the Gargano area, a special team of “Carabinieri” (a police unit of the Italian armed forces) discovered numerous bones, some of them in farmer’s boots, similar to human phalanges. Subsequently, they collected these bones and isolated the area, interrupting all activities and alerting the prosecutor. In fact, in 2010, a vehicle of a missing person was found near the same ravine. A team of forensic multi-specialists (forensic pathologist, anthropologist, and geneticist) promptly supported all operations. The team went deep into the ravine where bone identification became very difficult because of the commingled animal remains and other materials. Moreover, a large amount of mud rendered the recovery and identification processes tedious and difficult. During the recovery operations, numerous Kalashnikov bullets were found. After three days of work, no human skeletal remains were located.

The bones previously collected were transferred to the Institute of Forensic Pathology of the University of Foggia for a complete multidisciplinary investigation. Forensic investigations consisted of radiological, histological, anthropological, and genetic studies; all data excluded the presence of human phalanges. It was a sheep in boots!

In conclusion, this study demonstrates the importance of the forensic sciences to support investigative activities. A multidisciplinary approach is very important because it helped gather a variety of information and excluded the presence of human skeletal remains in the analyzed ravine. In the future, the same approach could be very useful to recover the corpses of 40 missing people in the Gargano area.

#### **Italian Mafia of Gargano, Forensic Multidisciplinary Approach, Concealed Corpses**

## E10 Three Cases of Traumatic Death?

*Costanza Filomena\**, Pisa 56126, ITALY; *Francesca Iannaccone*, Pisa 56123, ITALY; *Federica Gori, MD*, University of Pisa, Pisa 56100, ITALY; *Luigi Papi*, University of Pisa, Institute of Legal Medicine, Pisa 56100, ITALY; *Angela Pucci, MD*, University of Pisa, Pisa 56100, ITALY

**Learning Overview:** The goal of this presentation is to emphasize the difficulty of differential diagnosis between natural and traumatic deaths, especially when the circumstantial data is misleading. In such cases, histology proves to be useful in the identification of the real cause of death.

**Impact on the Forensic Science Community:** This presentation will affect the forensic science community by showing that although in some cases the manner of death may appear evident from circumstantial data, an underlying cause could be hidden, and only a thorough scene investigation and complementary analysis such as histology can reveal the true manner of death.

The first case regards a 31-year-old male found dead in his car at the bottom of river at a depth of 10 meters. An initial investigation by local police suggested the man lost control of the car, hit the barrier of the bridge, was thrown 25 meters from the impact point, and fell into the water. Toxicological analyses showed a high blood alcohol level (2g/l).

The second case, a 22-year-old male who was swimming in a camping pool, suddenly collapsed and fell underwater. Cardiopulmonary Resuscitation (CPR) was attempted by the lifeguard and, upon arrival of the ambulance, defibrillation was performed, without results.

The third case deals with a 21-year-old girl found dead by her motorcycle at the road side, still wearing her helmet. In all cases, postmortem Computed Tomography (CT) and toxicologic examinations were performed. CT scans revealed no signs of trauma, and toxicologic analysis was positive only in Case 1 for alcohol.

In Case 1, external examination revealed no remarkable findings. At section, heavy, edematous and congested lungs, with foam inside the bronchial branches, and a heavy heart were discovered. In Cases 2 and 3, external examination showed frothy fungus at the nostrils and oral cavity, and bilateral subconjunctival petechiae (Case 2) and perioral and zygomatic superficial abrasions, an oily, blackish material on the left knee, and zygomatic abrasions on both hands (Case 3). At section, both cases revealed heavy, edematous, and congested lungs, with foam inside the bronchial branches.

In all cases, Hematoxylin-Eosin (H&E) and Masson's trichrome staining were performed. Histologic examination documented massive lymphocytic infiltration associated with some histiocytes in full thickness sections from both ventricles (including endocardium and pericardium), and acute emphysema in Case 1. In Case 2, myocytic hypertrophy with myocardial disarray and fibrosis, especially in the antero-lateral wall of left ventricle (>5% of total heart samples), and some areas of myocardial bridging were found. In Case 3, diffuse myocytic hypertrophy with myocardial disarray (>5% of total heart samples), endomyocardial clefts, contraction bands, fibrosis, and some areas of myocardial bridging were discovered.

In two of the cases, death was attributed to hypertrophic cardiomyopathy, not, as hypothesized, a traumatic cause. Case 1 is still doubtful as the man could have died by drowning (significant pulmonary findings, such as edema, acute emphysema) or by the accident due to the high alcohol level or by myocarditis.

Sudden and unexpected death in otherwise healthy individuals is probably the most difficult differential diagnosis in forensic pathology, especially with the presence of misleading circumstantial data.<sup>1</sup> In such cases, histology, along with complementary investigations, is fundamental to identify the real cause of death.

Myocarditis may cause sudden death. The reported incidence is higher than expected in younger age groups (less than 35 years of age), with 12% of sudden cardiac deaths attributed to myocarditis in young Australians and 34.7% in a Chinese autopsy case study.<sup>2,3</sup> Therefore, myocarditis should be suspected in cases of sudden unexpected death in otherwise healthy young people. Representative sections from anterior, lateral, and the posterior wall of the atria and ventricles, as well as sections from the ventricular septum, should be submitted for microscopic examination. Histological diagnosis is defined by Dallas criteria. The presence of an inflammatory infiltrate in the myocardium (>14 lymphocytes/mm<sup>2</sup>) with degenerative and/or necrotic changes of adjacent cardiomyocytes are not typical of ischemic damage.<sup>4</sup>

Another frequent cause of sudden death, especially in young subjects (12–35 years old) is hypertrophic cardiomyopathy, characterized by myocardial hypertrophy, usually asymmetrical, located in the septum and, less frequently, left ventricle; recent studies have also described an involvement of the right ventricle in cases with a worse prognosis.<sup>5,6</sup> At histology, the most specific finding is myocardial disarray (in >5% of histologic samples), with myocardial bridging and interstitial fibrosis. Physiopathological mechanism of death is usually arrhythmia, due to unstable electric conduction through disarrayed myocardial areas.

Circumstantial data could sometimes be misleading; thus, a careful autopsic and histological investigation are fundamental, as highlighted in these three cases.

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### **Myocarditis, Hypertrophic Cardiomyopathy, Histopathology**

## E11 Child Sexual Abuse of Boys: A Six-Year Retrospective Analysis

Serena Maria Curti, MD\*, Sezione Medicina Legale DSSPP, Torino 10121, ITALY; Caterina Bosco, MD\*, Department of Public Health and Pediatrics, Turin, Torino 10126, ITALY; Sara S. Racalbutto, PsyD, Dipartimento di Pediatria d'Emergenza, A.O.U. Ci, Turin, ITALY; Elena Coppo, MD, Department of Pediatric Emergency, Turin, ITALY; Giancarlo Di Vella, MD, PhD\*, University of Torino, Department Public Health Sciences, Torino 10126, ITALY

**Learning Overview:** After attending this presentation, attendees will better understand the features of Child Sexual Abuse (CSA) perpetrated on male victims.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing tools to recognize a form of sexual abuse that is often difficult to identify.

The prevalence of CSA is dramatically high: in Europe it is estimated to be 13.4% for girls and 5.7% for boys.<sup>1</sup> However, the number of male victims is certainly underestimated for several reasons. Boys are usually abused by a caregiver, so they are not prone to disclose the abuse because of the strong affective bond between victim and perpetrator. Boys are also more reluctant to disclose the abuse for fear of reprisal or shame for being labeled as homosexual. Genital injuries are reported in less than 10% of the male victims, and few of them have been reported as highly suggestive of abuse.<sup>2-4</sup> The excellent healing capacity of children's tissues allows even serious genital lesions to go undetected even a few days after the trauma. Since the phenomenon is perceived as rare and few case series are available, literature about sexually abused boys is very limited and physicians have few tools to reach a correct diagnosis.<sup>5,6</sup>

In order to contribute to the knowledge of the topic, this study reports on the data collected from 2012 to 2017 in the multidisciplinary "Bambi" unit of the Pediatric Hospital "Regina Margherita" in Turin, Italy, composed by different health care providers trained in the assessment of child abuse. Among the 1,042 cases managed in this period, 494 were suspected cases of CSA. In 95 cases (19.2%), the victim of the suspected CSA was a boy. The cases highly suggestive of CSA were reported to the Judicial Authority (73 cases=77% of the suspected cases). For this group, the mean age of the boys was 6.9 years (range 2.5–15 years). Regarding the geographical origin of the victim's family, Italian children were predominant (51 cases), followed by Latin American (10 cases), African (7 cases), and eastern European (5 cases). In cases in which data was available, the family status analysis identified the same number of divorced and non-divorced parents (27 cases in both groups), while a small percentage of children resided in a community or with a foster family (7 cases). The most frequent suspected perpetrators were, with the same frequency, fathers and extra-familial people (both responsible for 33.8% of the cases), followed by other family members, such as victims' siblings (9.2%); 31.5% of the boys presented anogenital findings. These were mostly non-specific (erythema of the anal or genital tissues, 17 cases; anal fissures, 7 cases); only 2 cases revealed a finding highly suggestive of abuse (perianal laceration) and 87.7% of the boys did not present any skin injury. In the remaining cases, bruises and excoriations were observed in 6 cases, scars in 2 cases, and lacerated injuries in 1 case. In the suspicious cases managed by the "Bambi" unit in which, instead, the hypothesis of CSA was excluded after a careful multidisciplinary assessment (22 cases), the prevalence of negative anogenital inspection was slightly higher (77.2%). All the findings were non-specific (erythema, 1 case; anal fissures, 1 case; both, 2 cases; condyloma acuminatum transmitted by nonsexual contact, 1 case). There were no extra-genital skin lesions and the boys were older (mean age 7.9 years).

Despite the rarity of genital lesions in boys, health care providers must consider CSA as a possible differential diagnosis, especially when there is a disclosure or anomalous behavior of the child or the explanation of injuries by the caregivers is not consistent with the clinical findings. On the other hand, physicians who evaluate suspected cases of CSA must be familiar with pediatric diseases, pre-existing malformations, or "toilet trauma" that can resemble injuries caused by CSA in order to avoid over-reporting. For this reason, a multidisciplinary assessment conducted by a pediatrician, medical examiner, psychologist, and, eventually, urologist is recommended. This presentation provides attendees with better information concerning CSA perpetrated on male victims, which has not been extensively studied.

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### Child Abuse, Sexual Abuse, Male Victims

## E12 A Multiple Congenital Malformation in a Forensic Infant Autopsy

Francesco Lupariello, MD\*, University of Turin, Legal Medicine Section, Torino, ITALY; Serena Maria Curti, MD, Sezione Medicina Legale DSSPP, University of Torino, Torino 10121, ITALY; Janet B. Duval, MSN, Greensburg, IN 47240-8138; Giovanni Botta, MD, Department of Pathology, OIRM Sant'anna, Torino 10126, ITALY; Giancarlo Di Vella, MD, PhD\*, University of Torino, Department of Public Health Sciences, Torino 10126, ITALY

**Learning Overview:** After attending this presentation, attendees will understand the pathophysiology and presentation of Congenital High Airway Obstruction Syndrome (CHAOS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the methods necessary for diagnosing CHAOS at autopsy for forensic purposes.

CHAOS is a rare anomaly that is characterized by a partial or complete obstruction of the fetal upper airways. Its exact incidence is not known. The most common cause of CHAOS is the laryngeal atresia.<sup>1,2</sup> Other causes are laryngeal webs and cysts and tracheal agenesis or atresia. At prenatal Ultrasonography (USG), the fetus may be characterized by enlarged echogenic lungs, inverted or flattened diaphragm, and fetal ascites.<sup>3</sup> In fact, “the non-clearance of fluid from the lungs, due to obstruction, results in a dilated trachea and lung parenchymal hyperplasia. This further leads to compression of the great veins and right atrium leading to ascites. The compression of the esophagus due to a dilated trachea may lead to polyhydramnios.”<sup>2</sup> In addition, the heart may be centrally placed because of the compression of the enlarged lungs.<sup>4</sup>

This presentation reports on a singular case of CHAOS in a female infant in which congenital agenesis of the trachea and atresia of the esophagus led to her death shortly after delivery, despite intubation attempts.

A 26-year-old pregnant woman was transported by ambulance to the hospital because of intense uterine contractions. The information about the woman’s pregnancy was limited because she lived in another country during the previous months of her pregnancy. The only information for clinicians included: 33 weeks + 2 days of gestational age, no known maternal pathologies, and amniocentesis had not been conducted. The maternal weight, stature, and Body Mass Index (BMI) were, respectively, 63kg, 170cm, and 19kg/m<sup>2</sup>. The woman gained approximately 8kg of weight during the pregnancy and did not receive regular USG or clinical medical evaluations during pregnancy. The only fetal USG available (conducted 13 days before delivery) showed polyhydramnios, suspected esophageal atresia, and arthrogyposis.

Immediately after admission of the woman to the hospital, the physicians decided to perform an emergency cesarean section because the fetal cardiotocography was highly pathologic. At delivery, the baby weighed 1.7kg, was non-reactive to stimuli, atonic and cyanotic, and the APGAR score was 1, 0, 0. The heart rate was 30bpm. The neonatologist attempted to aspirate the secretions from the upper respiratory airways, but to no avail. Therefore, he immediately tried to intubate the baby, inserting the tube as far as the vocal cords, but was unable to advance the tube beyond that point. Finally, despite multiple intubation and resuscitation attempts, the baby died. The case was referred to the forensic pathologist.

On the gross examination, the heart was centrally placed, and both lungs were enlarged and characterized by two lobes. The larynx was normally developed, but the probe did not manage to reach the trachea due to a cartilaginous septum that was located between the larynx and the trachea. The distal part of the trachea and the bronchi were present, and the trachea (in correspondence with the carina) showed a trachea-esophageal fistula. On the other end, the esophagus was atresic. Indeed, the probe did not manage to go beyond the upper part of the esophagus. In addition, the distal part of the esophagus was present and connected to the trachea through the fistula mentioned above. There was also peritoneal effusion, a small stomach, unicornuate uterus, and a recto-cloacal fistula. The microscopic examination only revealed an accumulation of mucus in the alveoli. The death was due to the congenital tracheal malformation that did permit intubation. The manner of death was natural. Reviewing the literature, the pathologists understood that the baby was affected by a peculiar form of CHAOS in which a type 2 of congenital tracheal agenesis (Floyd classification) was associated with a type C esophageal atresia (Gross classification).<sup>5,6</sup> The combination of these congenital anomalies has not yet been described in the literature. This may generate difficulties in the interpretation of anatomical findings at forensic autopsy. For these reasons, this presentation should serve as a stimulus to improve knowledge in the forensic community regarding these types of congenital malformations.

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### Congenital High Airway Obstruction, Forensic Infant Autopsy, Multiple Congenital Abnormalities

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## E13 Penetration Injury From Child Sexual Abuse: A Systematic Literature Review

Patricia M. Speck, DNSc\*, Hoover, AL 35226; Caroline Concannon, BSN\*, University of Alabama at Birmingham School of Nursing, Birmingham, AL 35290-0001; Kimberly Peek, BSN\*, University of Alabama at Birmingham School of Nursing, Birmingham, AL 35242

**Learning Overview:** The goal of this presentation is to present a systematic literature review about full vaginal penetration of prepubertal children in published literature, exposing gaps in research. This presentation will impact the forensic science community by disentangling “it’s Normal to be Normal” (NtbN) categorization in Child Sexual Abuse (CSA) from “Full Penetration (FP).” At the end of this presentation, learners will: (1) identify two distinct care specialties for prepubertal children following sexual abuse—pediatric specialists and surgical specialists; (2) disentangle the legal and medical definitions of “penetration of the labia, however slight” from “full penetration,” respectively; and (3) distinguish disentanglement resulting in more questions about injury and long-term sequelae for prepubertal children experiencing full penetration.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by an analytical literature review about prepubertal child sexual abuse that was 3-fold: (1) provide a platform for pre-licensure students to learn one aspect of the research process; (2) distinguish clinical skills necessary for providers in the evaluation of FP of a prepubertal child; and (3) create a foundation for future study about the unique population characteristics in different settings

Legislation, describing penetration as “separation of labia, however slight” guides definitions in CSA research and prosecution. Consequently, scholars using the legal definition argue it is Normal to be Normal (NtbN).<sup>1-3</sup> However, children experience significant injury with FP (defined medically as piercing into a cavity), challenging the NtbN notion.<sup>4-7</sup> Literature reports injury classifications as normal/non-specific (NtbN) to definitive injury (FP), mixing “separation of the labia, however slight” from those experiencing FP past the hymen into vagina cavity and elsewhere.<sup>8</sup> Stud-designed selection bias currently prevents injury incidence disentanglement, resulting in the NtbN notion.

A systematic literature review of CSA injury articles was conducted. PubMed, CINAHL®, Scopus®, and Google® Scholar discovered 35 articles responsive to CSA injury. Non-responsive to FP, a reference list search for articles sufficient to analyze rare FP injuries found 4 articles.<sup>3,4,6,9-13</sup> Analysis revealed that CSA, defined as “separation of the labia, however slight,” rarely results in injury detection (1%-6%), but FP injuries require surgical evaluation (11%-18%) and repair (4%-12%) with significant pelvic-floor outcomes.<sup>2,4,6,14</sup>

The systematic literature analysis disentangled two distinct populations and outcomes. The CSA specialty clinic studies seldom identify FP injury in prepubertal children. When compared, emergency pediatric hospitals and surgical specialty practices experienced significantly more FP injury cases. Therefore, this systematic review rejects the notion that it is NtbN with FP. Future studies distinguishing normal findings and non-penetrating vulvar trauma from vaginal FP are necessary to fully disentangle data and measure long-term physical impacts of prepubertal FP.

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### Child Sexual Abuse, Penetration, Injury

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## E14 The Challenge of Diagnosing Child Abuse (CA): The Perks of a Multidisciplinary Approach

Francesco Lupariello, MD\*, University of Turin, Legal Medicine Section, Torino, ITALY; Sara S. Racalbutto, PsyD, Dipartimento di Pediatria d'Emergenza, A.O.U. Ci, Turin, ITALY; Elena Coppo, MD, Department of Pediatric Emergency, Turin, ITALY; Greta Cena, MD\*, Dipartimento di Scienze della Sanità Pubblica e Pe, Turin 10126, ITALY; Giancarlo Di Vella, MD, PhD\*, University of Torino, Department Public Health Sciences, Torino 10126, ITALY

**Learning Overview:** After attending this presentation, attendees will understand fracture patterns, which are highly suggestive of physical CA.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the importance of a careful, multidisciplinary evaluation in cases of fractures from suspected CA.

CA represents a diagnostic challenge due to its complex presentation. Although the consequences of failing to diagnose CA may be life-threatening, a wrong diagnosis may have devastating sequelae too.<sup>1</sup> Imaging plays a major role since there are fracture patterns, which are highly suggestive of physical abuse.<sup>2,3</sup> According to Kleinman, injuries with high specificity for abuse include Classic Metaphyseal Lesions (CMLs), rib fractures, especially posteromedial, scapular fractures, spinous process fractures, and sternal fractures.<sup>4</sup>

However, no fracture in itself is pathognomonic for child abuse.<sup>5</sup> Therefore, it is important to take into consideration other factors that can increase the likelihood of abuse. The most significant are: the age and the developmental stage (ambulatory or not) of the child, the consistency of clinical history provided by caregivers, the presence of multiple fractures in different states of healing, coexistence of other injuries suspicious for abuse (bruises, contusions or internal injuries), and the characteristics of the family.<sup>4</sup>

The goal of this presentation is to report findings of a retrospective analysis of CA cases in which the abuse was associated with one or more bone-fractures. This study reviewed all cases managed from January 2012 to December 2017 by the multidisciplinary unit (“Bambi”) dedicated to the evaluation of suspected abused children at the Ospedale Regina Margherita of Turin, Italy. The operators of the “Bambi” unit examined 1,042 cases of suspected CA. In 39/1,042 cases, there were one or more fractures, and in 25/39 cases, suspected abuse was confirmed. Among these 25 children, 16 were male and 9 female, the mean age was 18 months, and 20/25 were non-ambulatory. In 6 cases, high-specificity fractures were diagnosed (2 CML, 3 rib-fractures, and 1 in which CML and rib-fractures coexisted). In all of these 6 cases, the children were non-ambulatory. Bruises, abrasions, lacerations, and findings of abusive head trauma were respectively described in 9, 4, 2, and 5 cases. In 10/25 cases, the child’s kin modified the initial version regarding how the fracture occurred. Among the cases with high-specificity fractures, 2 children suffered a fall from less than 1 meter high, and in 2 cases, from more than 1 meter high. In 2 other cases, these data were not available.

According to literature, physical abuse is more likely to be the cause of a fracture in children who are not yet walking. In compliance with that, this study revealed that 20/25 children were not yet ambulatory, with a mean age of 18 months. Moreover, all 6 children who were diagnosed with fracture highly specific for abuse were non-ambulatory.<sup>4</sup>

Other than the specificity of the fracture and the age and developmental stage of the child, the history provided by the caregivers can make a fracture suspicious for child abuse. In 10/25 cases, the caregivers changed the initial history provided or gave discordant explanations. Among the 6 cases with high specificity fractures, the history related to 4 of these was inconsistent with the energy needed to cause the fractures. The kin provided either no detail of the dynamic of the event in 2 cases, or in 2 other cases, related a history of a low-energy event (fall from less than 1 meter).<sup>4</sup> In the study sample, males were more likely to suffer from CA than females, but in literature, no significant gender differences were observed in terms of prevalence of CA.<sup>6</sup> The literature states that CA occurs in all socio-economic groups and across all racial and ethnic groups.<sup>4</sup> In the present report, 20/25 children came from families of low ( $n=16$ ) or average ( $n=4$ ) socio-economic status.

In the present review, only 6/25 cases of CA had highly specific fractures, but in the other cases, the “Bambi” staff diagnosed CA on the basis of other relevant findings. This presentation should serve as a stimulus to heighten the importance of the combination of radiological, clinical, investigative, and social findings in order to achieve a reliable CA diagnosis with a multidisciplinary approach.

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### **Child Abuse, Fractures, Multidisciplinary Evaluation**

## E15 A Rare Case of Iatrogenic Skull Damage in a Newborn

Luigi Papi, University of Pisa, Institute of Legal Medicine, Pisa 56100, ITALY; Federica Gori, MD, University of Pisa, Pisa 56100, ITALY; Chiara Toni, MD, Pisa, ITALY; Sara Turco\*, University of Pisa, Pisa, Italy 56126, ITALY

**Learning Overview:** The goal of this presentation is to inform attendees concerning a very rare case of iatrogenic skull damage in a newborn due to cesarean section delivery without instrumentation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making a differential diagnosis of lesions of the newborn, which is mandatory due to medical malpractice and refund implications.

Compared with adults, newborn skull fractures are very rare: the relatively malleable nature of a newborn's skull and bones separated by sutures allows greater deformation and reduces susceptibility to fractures. Skull fractures of the newborn could be linear, usually restricted to one skull bone, complex or depressed, when part of the bone surface is displaced inward (ping-pong fractures).<sup>1</sup> Head fractures of the newborn are usually associated with the use of instrumentation, both in vaginally or cesarean section delivery.<sup>2-5</sup> In literature, there are also cases of newborn skull fractures after complicated delivery due to obstetric maneuvers without instrumentation.<sup>4</sup> Furthermore, some authors reported cases of spontaneous skull fractures associated with the compression of the head of the newborn by the mother's bony pelvis, 5<sup>th</sup> lumbar vertebrae, ischial spines, sacral promontory, and symphysis pubis.<sup>3</sup> Non-depressed compound skull fractures usually have a good prognosis and watchful waiting could be the only treatment, while depressed skull fractures are more frequently associated with blood flow hypoperfusion, brain edema, and tears or ruptures of vessels, which can lead to severe cerebral injury and the creation of epileptic foci.<sup>4,6</sup>

A 32-year-old woman came to the local hospital during her 29<sup>th</sup> week of pregnancy. She had a history of abortion during the 1<sup>st</sup> trimester. The current pregnancy went normally until the 29<sup>th</sup> week, when she was admitted in the obstetric ward for a premature delivery threat. Clinical and instrumental exams were regular, as were laboratory analysis, so the clinicians decided to dismiss the woman. A month later, she had a premature membrane rupture with limpid amniotic fluid. The woman was admitted to the hospital. She was at the 34<sup>th</sup> week of pregnancy. The baby was in a cephalic position, but electronic fetal monitoring showed a non-reassuring fetal heart rate tracing, so the clinician decided to proceed with a cesarean section. The baby was delivered without instrumentation. The surgeon described several attempts at delivery before managing to effectively extract the baby, who had already been deeply engaged in the pelvis. No evident trauma was registered. At the time of delivery, the newborn had an Appearance, Pulse, Grimace, Activity, Respiration (APGAR) of 6 after five minutes and a cardiac pulse of 130/min. The baby didn't cry and breathe, so clinicians started non-invasive ventilation, and, after one minute, they proceeded with mechanical ventilation. The baby presented with a head tumor and hematoma of the head, neck, and upper thorax. Neurologic examination at birth was not performed, because of the clinical condition of the newborn. Sonography of the head revealed a subependymal hemorrhage. During the intensive care recovery, the baby had an epileptic crisis treated with phenobarbital. Electroencephalography revealed pathologic electric activity, without clinical signs and with progressive improvement during the hospital stay. Head Magnetic Resonance Imaging (MRI) performed 18 days after birth showed bilateral parieto-occipital subdural hematoma. A cranial Computed Tomography (CT) made one month after birth revealed a bilateral parietal fracture and a compound skull fracture of the lambdoid suture and of the right coronal suture. In all of these fractures, the theca surface was not displaced inward. At present, the child has spastic quadriplegia. He maintains the sitting position in autonomy, while he needs help to reach and maintain the upright position. Walking is uncoordinated. He presents a cognitive deficit, for which he is followed by specialists.

This case is unusual and deserves attention as the newborn suffered from severe brain damage and severe neurologic sequelae. The goal of this presentation is to explain that even during cesarean section delivery without the use of instrumentation, skull fractures associated with severe brain damage from inadequate obstetric procedures can occur. From a medicolegal point of view, a differential diagnosis of iatrogenic and non-iatrogenic-spontaneous lesions is mandatory due to medical malpractice and refund implications.

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### Newborn Skull Fractures, Cesarean Section, Fetal Brain Damage

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## E16 Causes of Death in Patients Under the Age of 65 at the Bari Polyclinic

*Gianni De Giorgio\**, Section of Legal Medicine, Bari, ITALY; *Fiorenza Zotti*, PhD, Bari 70124, ITALY; *Eloisa Maselli*, MD, Bari 70122, ITALY; *Valeria Bruno*, MD, D.I.M., Bari, Puglia 70124, ITALY; *Valentina Ronco*, MD, Section of Legal Medicine D.I.M., Bari 70124, ITALY; *Davide Ferorelli*, Bari, ITALY; *Francesca Donno*, MD, University of Bari, Bari 70121, ITALY; *Alessandro Dell'Erba*, PhD, Risk Management Unit, Bari 70124, ITALY

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**Learning Overview:** The goal of this presentation is to identify the main causes of death in patients under the age of 65 years, demonstrating how some diseases are stratified by gender and age, probably in relation to specific risk factors, while others affect the study population almost transversely, placing legitimate doubt of environmental influences in the determining of events.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how some diseases are stratified by gender and age, while others affect the study population almost transversely. The stratification of mortality due to death is one of the most reliable epidemiological indicators aimed at monitoring the health status of a population.

According to the latest Italian National Institute of Statistics (ISTAT) report of 2012, beyond congenital pathologies and hematological malignancies that mainly concern childhood, in Italy, the main causes of death in subjects under the age of 65 years are cardiac diseases (16%) and tracheal, bronchial, and pulmonary neoplasms (12%). It is interesting to note that the heart diseases and the neoplastic pathologies—considered as a whole—are equivalent in the male sex, while cardiopathies are much less frequent in the female sex. Remaining in the field of oncological diseases, there is a clear preponderance of broncho-pulmonary and colon-rectal involvement in both sexes, with the addition of the high frequency of breast cancer in women.

The present analysis was conducted through a careful review of the ISTAT cards of patients who died in the Bari Polyclinic in 2017. The data were assessed in relation to the demographic characteristics of the patients (according to sex and age), the cause of death, and the department of hospitalization.

The patients who died in the Polyclinic in 2017, out of a total of 60,012 hospital discharge records, correspond to 1,200 units, of which 22% (268 units) were under 65 years of age. Analyzing the data regarding the sex of patients, among those under 65 years of age, there is a clear prevalence of mortality among male subjects compared to females (61% vs. 39%). The most reoccurrences, in relation to the intrinsic critical situations of patients at the time of admission, correspond to reanimation (33%) and internal medicine (11%). The stratification in relation to the causes of deaths emphasized the role of oncological pathologies in the determinism of deaths in the age group considered: in the Bari Polyclinic, 43% of the deaths in patients under 65 years of age are linked to neoplastic pathologies, followed by cardiovascular diseases (18%) and infections (18%).

Analyzing the neoplasms more specifically, the leading causes of death in the study population, leukemia and lymphomas, represent the most frequent neoplasms (20%), especially in the age group between 40 and 65 years of age; gastrointestinal and female genital-tract cancers are represented by 18% and 16%, respectively. Considering the different distribution of neoplasms in the two sexes, females have a higher mortality linked to tumors of the genital apparatus (41%) and colon-rectal and hematological (36% overall) tumors, while in the male sex it is interesting to note that, in addition to the prevalence of hematological tumors (20%), there is an exclusivity of pulmonary (14%) and hepatic tumors (12%).

Despite the small sample considered, the review conducted allows one to dwell on some peculiarities that partially differ from the national and international trend. First, there is an evident prevalence of neoplastic pathologies compared to heart disease, as well as a significant recurrence of causally related infections with deaths; moreover, in the field of neoplastic pathologies, the main role played by the diseases of the blood and of the blood and lymphatic organs is clear. A possible explanation for these particularities, in light of the risk factors of leukemia and lymphoma (solvents, ionizing radiation, in addition to drugs and congenital deficits), could result from further epidemiological studies related to environmental characteristics of the city and the province. Last, the pulmonary and hepatic neoplasms, exclusively in the case of the male sex, could be part of a still partially consolidated difference in lifestyle, relative to voluptuous habits.

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### Mortality, Patients, Neoplasms



### E17 An Analysis of Death at Long-Term Care Health Facilities in Japan

Alissa M. Shida, BA\*, *Department of Legal Medicine, Osaka, Osaka 545-0052, JAPAN*

**Learning Overview:** After attending this presentation, attendees will understand the risk factors in nursing homes in Japan and how preventing these risks factors would help that society.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reviewing the records of autopsy cases of nursing homes and helping the investigation that occurs in these facilities.

Japan's declining birth rate and aging population are severe, even when compared to the demographic situation in other developed countries. With the related explosion in the number of long-term care health facilities established to cope with increasing numbers of community-dwelling elderly, Japan is facing social issues concerning the recognition of unnatural deaths. Based on forensic autopsy cases at the Department of Legal Medicine, Osaka City University Medical School, the present study analyzed deaths at long-term care facilities from the perspective of social medicine.

The records of 26 autopsies performed between 2005 and 2015 at the Department of Legal Medicine, Osaka City University Medical School, Osaka, Japan, of deaths at long-term care health facilities were investigated regarding characteristics such as age, sex, time of death, type of long-term care facility, number of nursing care staff, state of care, cause of death (internal and external causes), medical history, and autopsy findings.

A total of 26 (1.2%) of the 2,211 autopsies performed at the Department of Legal Medicine, Osaka City University Medical School between 2005 and 2015 were of deaths at nursing homes, ranging from approximately two to four cases per year. Women and individuals in their 80s represented 65% ( $n=17$ ) and 46% of cases, respectively. By facility type, most cases were from special nursing homes for the elderly (publicly operated facilities that provide nursing and custodial care services to elderly people requiring everyday care (65%;  $n=17$ ), followed by private residential care homes (privately operated homes for the elderly that provide nursing care services to relatively self-reliant elderly people;) (34%;  $n=9$ ). There was also one case each from an intermediate care facility (facilities aimed at helping residents  $\geq 65$  years old to return home) and a private nursing care home (privately operated residential facilities with 24-hour staffing for elderly people requiring nursing care).

For both internal and external causes, more deaths occurred at night (69%;  $n=18$ ) due to lack of staff compared to daytime (two staff). Causes of death were external in 15 cases and internal in 11 cases. After internal causes, the most common causes of death were blunt force trauma followed by drowning, asphyxia, and fire. The most common internal causes of death were cardiovascular disease (46%), pneumonia (18%), and malnutrition (18%). Known illnesses diagnosed before death included hypertension (34%;  $n=9$ ), dementia (30%;  $n=8$ ), cerebral infarction (23%;  $n=6$ ), and diabetes mellitus (11%;  $n=3$ ). Conversely, central nervous system diseases (46%;  $n=12$ ), circulatory conditions (42%;  $n=11$ ), and digestive system disorders (42%;  $n=11$ ) were only identified during autopsy. The predominant cause of blunt force trauma was falling, which is the most common external cause of death (60%;  $n=9$ ). Among these cases, 56% ( $n=5$ ) were to the head. Suspected negligence was involved in three cases of bathtub drowning, three cases of wheelchair falls, two accidents during assistance with activities such as changing clothes, and one case of aspiration and asphyxia while eating. Of these nine cases of suspected negligence, 78% ( $n=7$ ) occurred when staff was not in attendance. The present findings suggest that with the increasing establishment of long-term care facilities in response to the declining birth rate and aging population, systems of facility staffing and medical treatment by professionals such as doctors and nurses should be verified.

#### Legal, Health Care, Aging Population



### E18 The Faithless Nurse: A Strange Case of Hyperthermia

*Francesca Maglietta, MD\**, Department of Forensic Pathology, Foggia 71122, ITALY; *Lorenzo Spagnolo, MD*, Department of Forensic Pathology, Foggia, Foggia 71122, ITALY; *Michela Ferrara, MD*, Department of Forensic Pathology, Foggia 71122, ITALY; *Dania De Carlo, MD*, Ospedale Colonnello D'Avanzo, Foggia 71100, ITALY; *Giuseppe Davide Albano, MD*, Foggia 71121, ITALY; *Mauro A. Ciavarella, University of Foggia, Department of Forensic Pathology, Foggia 71121, ITALY*

**Learning Overview:** The goal of this presentation is to illustrate the risk of developing serotonin syndrome, particularly with cases involving administration of narcotics, such as methadone, in conjunction with other serotonergic agents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enhancing awareness regarding co-administration of serotonergic agents among heroin abusers who receive methadone maintenance therapy. This presentation will also stress the importance of developing the knowledge of mechanisms leading to hyperthermia and related to methadone consumption that have proven to be the culprit of serotonin syndrome.

Toxicological investigations are essential in identifying narcotics opioids overuse in suspected patients and should be performed routinely, but, unfortunately, the metabolism of methadone demonstrates extreme interindividual variation. This results in physicians not always performing a correct diagnosis in cases of serotonin syndrome. Clinical presentation includes a triad of autonomic instability (tachycardia, tachypnea, and hypertension), altered mental status (agitation and coma), and neuromuscular overactivity (increased tone). In life-threatening conditions, severe hyperthermia, hypertension, and tachycardia may lead to frank shock and coma. Typically, symptom onset occurs rapidly within hours of an increased dose of a serotonergic agent. Data from the Centers for Disease Control and Prevention (CDC) reveal that the United States opioid overdose epidemic continues to worsen, and guidelines on the prescription of opioids in chronic pain situations do not recommend the simultaneous administration of narcotics with other serotonergic agents.

In the hot summer of 2014 in Foggia, in the south of Italy, many inmates of the local prison experienced illness due to high temperatures. A 51-year-old man was found unconscious in the bed of his cell, wearing nine sweaters and three pairs of pants, covered with a wool blanket. The windows and the blind (type of door) of the cell were closed and the jail cell temperature was 29° Celsius. The man was rescued, transported to the hospital, and died shortly after his arrival at the emergency department from a malignant arrhythmia. The anamnestic data revealed that the man was a heroin addicted in daily treatment with methadone, paroxetine, and quietapine, and suffered from both Klinefelter Syndrome with hormone replacement treatment and from an anxious-depressive disorder. Approximately four hours after death, his rectal temperature was 41° Celsius. The external examination conducted on the cadaver was negative for signs of acupuncture or traumatic injury. Autopsy of the convict was performed after two days, with a complete biological sampling for toxicological purposes. Not surprisingly, all organs exhibited unspecific gross alterations and the immunohistochemical staining of kidney samples revealed some lumens and epithelial cells of proximal and distal renal tubules positive to anti-myoglobin antibody. Serum samples, collected at the time of hospitalization, detected positivity for paroxetine, quietapine, and methadone. However, the kidney and liver failure made it impossible to quantify the rate of recruitment due to a slowdown in the reduction in the elimination rate.

The results of criminal investigation by the police revealed that the nurse who took care of him had given him two doses of methadone, claiming that the first dose was inadvertently dropped while being taken in the cell. This scenario was reconstructed. Positivity in serum of paroxetine, a drug usually taken by the prisoner, justified its predisposition. By virtue of the inhibition that this SSRI has on cytochrome CYP2D6 and 3D4, it allowed a greater sensitivity to the double dose of methadone, favored the rapid development of a serotonin syndrome associated with coma and malignant hyperthermia, and allowed the settlement and orientation of the known inconclusive gross and histological findings for the syndrome.

#### **Methadone, Serotonin Syndrome, Hyperthermia**



### E19 A Tale of Two Sisters: A Case Review of a Suicide Pact Between Adult Siblings

Meryle A. Dotson, MA\*, Hillsborough County Medical Examiner's Office, Tampa, FL 33616; Mary Mainland, MD, Hillsborough County Medical Examiner's Office, Tampa, FL 33617

**Learning Overview:** After attending this presentation, attendees will be more familiar with a case of a successful suicide pact involving two adult siblings by ingestion of tramadol, doxylamine, diphenhydramine, and hydrocodone.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a unique scenario in which a thorough investigation of the death scene revealed evidence of premeditation and cooperation between two sisters who chose to enter into an agreement to end their lives together.

This information will assist law enforcement and medicolegal investigators by contributing to the understanding of the manner in which a scene investigation can reveal the sequence of events leading to the simultaneous deaths of two individuals. In this case, a multidisciplinary approach to death investigation was imperative. A detailed examination of the scene, thorough autopsy examination, and toxicological analysis permitted investigators to reasonably exclude the deaths to be results of a homicide-suicide.

A suicide pact is defined as a mutual agreement between two or more people to kill themselves, usually at the same time and location.<sup>1</sup> Suicide pacts are rare occurrences and represent less than four percent of all suicides. The frequency of successful suicide pacts is noted to be highest in Japan, where suicide pacts are more often reported among lovers.<sup>2</sup> In the United States, suicide pacts have been reported among aging, married couples as well as among siblings who face separation due to the ailing health of another sibling.<sup>1</sup> When investigating cases of reported suicide pacts involving two decedents, a thorough investigation of the death scene is imperative in determining whether the deaths of both individuals are a result of a successful suicide pact as opposed to a homicide-suicide. An essential component of a suicide pact that can be identified at the death scene is evidence of consent by both parties.<sup>3</sup> This presentation will present circumstances of the scene investigation and autopsy findings for an unusual case in which a successful suicide pact was carried out between two adult sisters who entered into an agreement to end their lives together, likely due to the failing health of the older sister.

The deceased in this case are a 61-year-old woman and her 52-year-old sister who resided together in an apartment in a residential Florida neighborhood. The older sister was previously diagnosed with multiple medical conditions, and her younger sister served as her primary caretaker. Neither sister had been seen during the week prior to being found deceased. Apartment complex staff noted a foul odor emanating from the residence and contacted law enforcement. Law enforcement forced entry into the residence and located the bodies of both siblings in states of decomposition on a bed in a rear bedroom. A table in the corner of the room contained two personalized memory boxes, urns, and pre-need contracts for cremation authorized by each sibling. A handwritten letter in the bedroom stated that the younger sister was not willing to continue life without her older sister and provided explicit instructions for final disposition of their bodies. The letter indicated that the sisters had no other surviving family members, and that they "choose euthanasia." The letter contained the signatures of both sisters. There was no apparent trauma to the bodies. Multiple empty juice bottles, empty pudding cups, and two empty vials bearing the initials of each sister were recovered from a plastic bin beside the bed. After subsequent investigation of the circumstances, foul play was not suspected.

When investigating a death scene of a potential suicide pact, investigators should keep in mind the possibility that the deaths may be the results of a homicide-suicide. Due to their rare occurrences, suicide pacts are infrequently reported in the literature. However, the cases that are published describe several hallmarks of a suicide pact that can be identified at the death scene.<sup>3</sup> Consent and understanding among all parties, detailed planning performed in secrecy, and execution of the pact in privacy are components of a suicide pact that cannot be identified by autopsy alone. This case study contributes to the documentation of suicide pacts between siblings in the United States and illustrates that a thorough, multidisciplinary investigation of the death scene is necessary in order to confidently determine the of manner of death as suicide by consensual means.

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#### Double Suicide, Suicide Pact, Death Scene Investigation



### E20 A Case of Strangulation With a Cable Tie: Homicide or Suicide?

*Omar Bonato, MD\*, University of Ferrara, Ferrara 44121, ITALY; Elena Lucenti, MD, University of Ferrara, Ferrara 44121, ITALY; Mauro Coppone, MD\*, University of Ferrara, Ferrara 44121, ITALY; Erica Bacchio, MD, University of Ferrara, Ferrara 44121, ITALY; Chiara Marini, MD, University of Ferrara, Ferrara 44121, ITALY; Letizia Alfieri, MD, University of Ferrara, Ferrara 44121, ITALY; Rosa Maria Gaudio, University of Ferrara, Ferrara 44121, ITALY; Lorenzo Marinelli, AOU Arcispedale Sant'Anna, Ferrara 44124, ITALY; Margherita Neri, MD, PhD, University of Ferrara, Ferrara 44100, ITALY*

**Learning Overview:** The goal of this presentation is to expose a case of self-strangulation suicide in which plastic cable ties were used, analyzing and drawing attention to features that can be found in a forensic investigation in cases of suicidal and homicidal ligature strangulation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that, in a case of ligature strangulation, the differentiation between homicide and suicide can be difficult. This presentation can provide important support for the management of similar forensic cases.

Self-strangulation by ligature is an uncommon occurrence in forensic practice. In fact, strangulation cases are primarily homicides, with a few cases of sexual asphyxia or suicide.

A dead body of a 32-year-old male of Moldavian origin was found by the police at 1:40 a.m. along an isolated country road next to a truck. The victim was found in a supine position with a single plastic cable tie around the neck, with the knot (locking head) on the right posterolateral cervical region. Additionally, three cable ties around the ankles were documented. On the right side of the neck, a metal pincer was found, with the cutting edges that clamped a cutaneous fold immediately above the ligature. The subject had no history of psychiatric disorders, but financial problems were reported. Police officers initially suspected homicide.

A medicolegal autopsy was conducted the next day. At external examination, there was marked facial congestion with massive petechial hemorrhage in the face and in the conjunctivae. The ligature-produced furrow was evaluated after the ligature was removed; it was horizontally oriented, pale, deep, and encircled the neck completely. The pattern of the internal surface of the cable tie was reproduced in the furrow. The right cervical region, where the pincer was found, showed two small superficial injuries of the skin, with hemorrhagic infiltrates. No other external injuries were noted on the body.

Internal examination of the neck showed some hemorrhages of the left thyrohyoid muscle, thyroid cartilage, hyoid bone, base of the tongue, palatine tonsil, and to the cervical prevertebral fascia. No fractures to anatomical structures of the neck or elsewhere were found. All organs were congested, with petechiae on serosal surfaces of epicardium and visceral pleura. There were no other significant findings at autopsy. Histopathological examination of the skin on the furrow showed the vitality of the wound. No signs of struggle or other antemortem injuries were found. The ligature at the ankles were loosely bound and were easily released. The death was deemed a suicide by ligature strangulation.

There have been only a few previous reports in the literature regarding self-strangulation suicide in which cable ties were used. More often-described case reports involve the use of belts, ropes, and scarves. Furthermore, this study presents an unusual case of suicide in which plastic cable ties were used both for self-strangulation and for self-restraint of the ankles to prevent any possibility of getting free. However, the neck wounds made with the pincer to undo the ligatures appear unusual because, in similar cases, injuries indicating an attempt to relieve applied pressure are not seen, underscoring the rapid loss of consciousness.

In cases of ligature strangulation, forensic pathologists have to consider all the circumstances (crime scene investigation, historical data, autopsy, and histopathological examination) in order to differentiate between homicide and suicide, especially when the crime scene investigation is ambiguous.

#### **Strangulation, Suicide, Autopsy**



### E21 The Evolving Suicide Note

*Imron G. Ramos, BS\*, Clovis, CA 93619; Ismail M. Sebetan, MD, PhD\*, National University, La Jolla, CA 92037-1011; Paul Stein, PhD\*, National University, La Jolla, CA 92037*

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**Learning Overview:** After attending this presentation, attendees will understand the importance of social media and electronic devices as an evolving entity for medicolegal death investigation compared to the age-old method of examining handwritten suicide notes, offering important legal implications regarding the manner of death of suicide vs. homicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a detailed review of data collected over a ten-year period (2007–2016), reviewing all cases in which the manner of death was documented as a suicide. Case-related data was also obtained from the Community Regional Medical Center in Fresno, CA, and is the only Level 1 Trauma Center between Sacramento and Los Angeles, thus treating and serving surrounding counties in Central California.

Most research regarding suicide and the notes left behind by the deceased begin by reviewing the medicolegal death investigation file, coroner's inquest, and/or medical examiner's report. Medicolegal death investigators are responsible for recording and documenting suicide notes found during their investigation. In this study, suicide notes were divided into media and non-media types. Non-media suicide notes are handwritten. Media suicide notes are generated by a computer, cell phone, audio, video, social media platform (i.e., Facebook), or any other type of electronic communication device, and whether the suicide note, if left on a cellular phone, was a text message or an instant message.

This research project had four objectives to determine if there were notable difference between non-media and media suicide notes and how the notes were left. Objective 1 was to determine if people are leaving significantly less non-media suicide notes compared to media suicide notes. Objective 2 was to determine if younger people are leaving significantly more media suicide notes than older people. Objective 3 was to see if there is a significant increasing rate of media suicide notes over each year for the past ten years. Objective 4 was to determine if personal cell phones are the main source for leaving media suicide notes compared to all the other electronic devices

Of the 881 total suicide victims, 212 left a suicide note (24%). Out of the 212 suicide notes left, 162 (76%) were non-media notes and 54 (24%) were media suicide notes. Of the media suicide notes left, 87% were left by cell phone over the entire ten-year span of collected data. However, in 2007–2011, 17 media suicide notes were left, and from 2012–2016, 37 media suicide notes were left, reflecting an increase of 217%. Although the suicide note rate has remained relatively consistent over the ten-year span, the frequency of social media notes has increased when compared to non-media suicide notes over the most recent five-year time frame.

These results show that media suicide notes are being left in a higher frequency than non-media notes and that cell phones are the preferred mode of leaving these notes. This apparent change over the manner in which suicide victims convey their innermost thoughts to those left behind may be indicative of the population statistics that showed in 2016, 28.6% of the population were under 18 years of age and 11.8 % were 65 years and older, with 59.6% between these age groups. Thus, the use of social media for leaving behind suicide notes, by a younger population more familiar with the internet and those tools, would not be unusual. It also suggests that there may be suicide ideations on the social media links.

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#### Suicide Notes, Investigation, Forensic Sciences





### E22 Determining the Accuracy and Suitability of Measurement Apps for Crime Scene Documentation

Victoria Zeger\*, West Melbourne, FL 32904; Christina A. Malone, MSFS, Defense Forensic Science Center, Forest Park, GA 30305

**Learning Overview:** After attending this presentation, attendees will better understand the current capabilities of several mobile device measuring software applications (apps) that have the potential for obtaining measurements when documenting a crime scene.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the benefits and limitations of the forensic use of mobile device measuring apps and the potential for such apps to be a low-cost, user-friendly, and efficient method of collecting measurements at a crime scene.

The spatial location of evidence drives crime scene documentation, requiring the measurement of numerous distances during the course of a criminal investigation. One major advantage of crime scene documentation involves placing the judge and jury into the scene through reconstruction techniques. Traditional reconstruction techniques are laborious and time consuming, while automated methods for capturing distance measurements (e.g., total stations and 3D laser scanners) may be cost restrictive. An efficient, portable, cost-effective device that can match the accuracy of a tape measure is ideal for crime scene documentation.

With the prevalence of mobile devices, the development of apps has flourished. The advancements in technology have resulted in the inclusion of augmented reality into these applications, allowing real and virtual worlds to interact in real time. Measurement apps can generate distance measurements either through augmented reality or point-and-shoot methods. However, both methods involve a set of algorithms that utilize data from sensors to output measurements. Image sensors and display sensors are major contributors of input data and offer technical limitations to the application and variability among devices. The sensitivity of the technology in the mobile devices and the precision of the data type used in the application are critical for accurate outputs.

Three mobile phones (Apple® iPhone® 7 Plus, Samsung™ Galaxy Note® 3, and Amazon® Fire®) were selected for use in this study due to their availability. Pertinent information on each phone was documented due to the potential impact of the operating system and sensor size/type when collecting distance measurements through each phone's camera. A series of known distances was established through hand measurement with a tape measure. Repeated distance measurements were then collected using each of three apps (AirMeasure, Smart Measure, and 3D Measure) specific to the phones operating systems (iOS®, Android®, and Fire® OS, respectively). Factors that were presumed to affect the accuracy of the measurements, such as distance from which the measurement was taken, lighting, angle, and shape/size of objects, were assessed. Statistical analyses were conducted to determine which apps produced the most consistent and accurate results compared to the hand-measured distances. The data collected, combined with an assessment of the ease of use for each app, demonstrates the benefits, limitations, and overall suitability for using mobile measuring apps for crime scene documentation.

*The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army (DA) or the Department of Defense (DoD). Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, the Defense Forensic Science Center (DFSC), the United States Army Criminal Investigation Command, the Office of the Provost Marshal General (OPMG), the DA, or the DoD.*

#### Measurements, Documentation, Crime Scene



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### E23 Observation, Explanation, and Testing in Forensic Science Using a Death Investigation Active Learning Assignment

*Trevor I. Stamper, PhD\*, Purdue University, West Lafayette, IN 47907; Krystal R. Hans, PhD, Delaware State University, Dover, DE 19901*

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**Learning Overview:** After attending this presentation, attendees will understand some principles for instructing students in teaching scientific observation, explanation, and testing in an undergraduate education setting using forensic examples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an understanding on how framing the scientific method in the proper context can allow students to better understand how the forensic process is itself scientific, from the collection of the trace to the use of that trace in trial.

Traditional forensic science textbooks offer little guidance on how to approach teaching the salient features of the scientific method—observation, explanation, and testing—as an inherently *forensic* endeavor. However, without an appreciation of the scientific method, teaching forensic subdisciplines is more science appreciation than science application. Over four iterations of Purdue’s Entomology (ENTM) 22810: Forensic Investigation course, students were provided detailed scientific method active learning assignments, but failed to connect these assignments to the scientific method and forensic science subdisciplines. It was realized that this was the result of using non-forensic examples or forensic examples lacking a trackable process with a discernable beginning, middle, and end. To resolve these disconnects, an active learning assignment was created from a real *New York Times* article series of a 1922 double death investigation. The current assignment has been used in four iterations of ENTM 22810: Forensic Investigation.

This case study demonstrates the importance of observation, explanation, and testing in a forensic science context, and also allows students an approachable way to grasp the concepts of relevance and anomalous phenomena. Further, students are trained to differentiate facts, conjectures, and assumptions from each other. Students assess how these different elements impact the possible explanations for the deaths of Mr. and Mrs. Jackson as the case matures. This is accomplished because students organize the various explanations using a supported argument format. The assignment further provides students with an inquiry-based structure to objectively evaluate observation events not previously presented in the forensic context.

This case highlights the actions of the first forensic toxicologist in the United States, Alexander Gettler, while at the same time exploring the impact that historical events, such as prohibition, have on cases. Teaching methods are highlighted and physical examples of the *New York Times* articles and active learning assignments are presented for attendees to review. Students enrolled in this course completed surveys prior to this assignment to serve as a baseline measurement of knowledge regarding crucial concepts: anomalous phenomenon, relevance, observation, explanation, testing, and understanding the difference between facts, conjectures, and assumptions. At the end of the semester, after completing the active learning assignment, students are provided with a post-assignment survey. Student learning was assessed by comparing the students’ abilities to decipher these concepts from the beginning to the end of the semester.

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#### Undergraduate Education, Scientific Methods, Case Analysis



## E24 The Development of a Flexible Algorithm for Substance Identification Using Mass Spectrometry

*Samantha A. Mehnert\**, West Virginia University, Morgantown, WV 26506; *Brandon D. Lowe*, St. Vincent College, Latrobe, PA 15650; *J. Tyler Davidson*, MS, West Virginia University, Morgantown, WV 26505; *Glen P. Jackson, PhD*, West Virginia University, Morgantown, WV 26506-6121

**Learning Overview:** After attending this presentation, attendees will better understand the value of ion correlations in mass spectrometric analyses through the demonstration of a dynamic algorithm that provides higher discriminatory power and increased confidence in mass spectrometric compound identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a mathematical model for compound identification from mass spectrometric data that is more accurate and more precise than current static/discrete methods. The application of a more selective algorithm will decrease the incidence of false positives and further assist with the identification of unknown compounds.

It is hypothesized that a mathematical model that employs the covariance between ion abundances will provide a more discriminatory algorithm with improved compound identification rates relative to a model that does not take into account the covariance between ion abundances.

Current mass spectrometric methods of substance identification use a static algorithm to determine the identity of a substance. Existing algorithms compare the relative abundance of each peak within a given spectrum to those of discrete reference spectra in a library. However, the variance in ion abundances for this type of comparison is generally around  $\pm 20\%$ , which can result in false positives for substance identification. An algorithm that can adapt to varying relative ion abundances using a continuously variable model will provide a more selective and more accurate identification algorithm than the current discrete methods.

Replicate measurements of various chemical standards show that ion abundances within replicate mass spectra of the same compound are not independently variable, but correlated or anti-correlated with coefficients of correlation as strong as an  $R^2 \geq 0.8$ . Two databases were developed to assess the accuracy and selectivity of this dynamic model. The first database consists of five illicit drugs, and the second database was comprising eight n-alkanes. The ion abundances were normalized to the base peak and the 15–16 most abundant ions were selected to be the dependent variables within the general linear models. The general linear models were constructed within SPSS statistical software using 90% of the spectra in each database. The remaining 10% of the spectra were used to assess the accuracy of the model. The model essentially predicts ion abundances at each  $m/z$  value, and these predicted abundances are then compared to the measured spectra using Pearson product-Moment Correlations (PPMCs). The significance of different PPMC's were compared through z-tests on Fisher transformations of the PPMC's.

The dynamic/continuous model was able to distinguish between the five illicit drugs with ease. The model provided significantly better ion abundance predictions for true positives than for true negatives, which is a requirement of a successful model. Consecutive n-alkanes (e.g., C16 vs. C17) were difficult to discriminate using comparisons of the PPMC's, so additional statistical analyses were performed to provide meaningful assessments for spectral identifications.

### Dynamic Algorithm, Mass Spectrometry, Ion Correlations



### **E25 A Qualitative Analysis of Human Growth Hormone (HGH) by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) and Complementary Techniques**

*Breanne Hamlett\*, Defense Forensic Science Center, Forest Park, GA 30297; Natalie Howard, BS, Defense Forensic Science Center, Forest Park, GA 30297; Anna L. Deakin, MS, Defense Forensic Science Center, Forest Park, GA 30297; Brent M. Allred, PhD, Defense Forensic Science Center, Forest Park, GA 30297*

**Learning Overview:** The goal of this presentation is to introduce attendees to the analysis and identification of protein-based molecules used in conjunction with controlled steroids, such as HGH. Liquid Chromatography/Mass Spectrometry (LC/MS) and LC/MS/MS methods on intact and digested proteins will be discussed, as well as the theory and protocol for commercial Enzyme-Linked Immuno-Sorbent Assay (ELISA) kits specific to the protein of interest.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a new series of techniques for highly specific identification of a protein-based hormone, such as HGH.

The United States Food and Drug Administration states that HGH and other related proteins are only approved as a prescription for a limited number of conditions and are prohibited for use as an anti-aging product, body-building supplement, or for muscle enhancement. Independently, United States Army Regulation 600-85 prohibits the use of prescription medications for anything other than their intended purpose or possessing a medication without a prescription. Military investigating agents often submit for analysis HGH and related proteins along with other controlled steroids. When submitted substances are suspected to be testosterone-related steroids, there are routine small molecule assays suitable for their identification. However, due to their complexity and size, proteins such as HGH must be analyzed using alternative methodologies.

In this study, HGH was analyzed using two LC/MS/MS-based methods, as well as sandwich ELISAs. The intact HGH protein was analyzed via LC/MS using a reverse phase, highly porous column. The intact protein demonstrated a repeatable retention time and characteristic multiply-charged ions from which the whole molecular weight of intact HGH was calculable. The ELISA-based assay successfully bound the folded HGH protein as indicated by the colorimetric response, with no observed cross-reactivity. Last, the trypsin digested peptide sequencing assay provided the greatest specificity. Predictable peptide fragments, unique to HGH, were obtained via a classic trypsin digestion of the protein, then were sequenced using LC/MS/MS fragmentation. An average of 85% coverage of the protein's expected peptides was observed. The ten specific HGH peptides that were further fragmented to acquire amino acid sequencing data resulted in the unique identification of HGH when compared to more than 139 million sequences in the National Center for Biotechnology Information (NCBI) protein database. Each of the three experimental methods resulted in complementary and orthogonal proteomic data, thereby making the identification of HGH highly specific.

Once an identification protocol was developed, the stability of HGH was investigated, since submitted samples can be in various states of degradation, potentially resulting in the misidentification of the protein. Numerous storage conditions were probed, including as a lyophilized powder or in assorted diluents, at different temperature ranges (up to 37°C), and over various time scales (up to three months).

For up to one month, no samples, regardless of storage conditions, showed degradation in the whole molecule LC/MS method, the trypsin digested LC/MS/MS method, or the ELISA method.

While the pros and cons of each method should be considered when creating an overall method for protein identification, the complementary information gained from each technique creates an overall highly specific method for the identification of HGH. The introduction of probabilistic language into forensic chemistry chemical identification reports will be discussed.

#### **Human Growth Hormone, Protein Identification, LC/MS Method Development**



**E26 WITHDRAWN**



## E27 Flubromazepam: Synthesis and Characterization of Positional Isomers for Forensic Analysis

*Evelyn S. Ligon\**, Atlanta, GA 30318; *Jason R. Nawyn, MSFS*, U.S. Army Criminal Investigation Laboratory, Forest Park, GA 30297; *Lonnie V. Jones II, BS*, U.S. Army Criminal Investigation Laboratory, Forest Park, GA 30297; *Stefan A. France, PhD*, Georgia Institute of Technology, Atlanta, GA 30332; *Brent M. Allred, PhD*, Defense Forensic Science Center, Forest Park, GA 30297; *Daniel V. Reinhardt, PhD*, U.S. Army Criminal Investigation Laboratory, Forest Park, GA 30297

**Learning Overview:** The goal of this presentation is to demonstrate a complementary application of organic and analytical chemistry for the forensic analysis of Novel Psychoactive Substances (NPS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing: (1) useful reference data for flubromazepam, a designer benzodiazepine of interest, and its 11 positional isomers; (2) a synthetic scheme to prepare pure standards of additional benzodiazepine positional isomers; and (3) a baseline analytical protocol to facilitate future forensic analysis, whether through new reference spectra or through predictions derived from spectral pattern recognition.

Benzodiazepines constitute a class of sedative, depressant, and relaxant compounds, many of which are controlled under Schedule IV of the United States Code Controlled Substances Act. Positional isomers of benzodiazepines are nearly identical structurally and are expected to exhibit similar bioactivity as a result. But due to the technically legal status of various benzodiazepine isomers, as the definition of “analog” in the Code of Federal Regulations does not apply to Schedule IV benzodiazepines, they represent potentially attractive substitutes for drug abuse. Few of these positional isomers are represented in scientific literature and, consequently, the reference analytical data or commercial reference standards necessary for accurate identification are not available to forensic chemists. Therefore, if a positional isomer of a scheduled benzodiazepine were ever presented as a legal alternative by a distributor, positive identification would prove challenging due to the lack of characteristic reference data.

In this study, flubromazepam, a recognized designer benzodiazepine since 2012, was targeted for synthesis and characterization due to its potential for federal scheduling. Currently, flubromazepam is not federally scheduled within the United States, but the strict laws imposed on military personnel to ensure operational readiness still make this a compound of active interest to the United States Department of Defense. Additionally, flubromazepam has appeared as evidence submitted in forensic casework with regard to investigations concerning drug-related civilian crimes and military operational readiness violations.

This project was divided into two phases: synthesis and characterization. First, a uniform synthetic method was developed to prepare purified reference materials of each positional isomer of flubromazepam for which the positions of aromatically bound bromine and fluorine were varied. Traditional methods are not optimal for these positional isomers, as they are low-yielding and non-regioselective, leading either to amounts of precursor so small as to prevent completion of the synthesis or to mixtures of isomers that are difficult to separate. Though the chemistry employed in this study would likely not be used by typical drug distributors, sufficient pure quantities of each isomer were successfully obtained for analysis.

Second, the corresponding analytical reference spectra for each positional isomer of flubromazepam were collected. The structural identification of synthesized flubromazepam isomers were validated primarily using High Resolution Accurate Mass Spectrometry (HRAMS) and both Proton and Carbon Nuclear Magnetic Resonance ( $^1\text{H}$ -/ $^{13}\text{C}$ -NMR). Isomers were then characterized using traditional forensic analytical techniques such as Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography/Mass Spectrometry (LC/MS), and Gas Chromatography/solid phase Infrared Spectroscopy (GC/IR). From this data, an analytical scheme was developed to accurately identify and differentiate each positional isomer of flubromazepam. Additionally, pattern recognition by NMR and IR was established to enable predictive analysis of unknown positional isomers that may appear in future forensic cases.

### **Flubromazepam, Positional Isomer, Novel Psychoactive Substance (NPS)**



### E28 Sudden Infant Death in a Newborn From Drug-Dependent Parents: The Utility of Hair Analysis

Costanza Filomena\*, Pisa 56126, ITALY; Silvio Chericoni, University of Pisa, Pisa, ITALY; Fabio Stefanelli, University of Pisa, Pisa, ITALY; Chiara Toni, MD, Pisa, ITALY

**Learning Overview:** The goal of this presentation is to emphasize the usefulness of hair analysis to identify chronic exposure and life-threatening situations for children of drug-dependent parents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing specific neonatal data that are lacking and that the interpretation of hair results with respect to systemic or only external exposure is particularly important for an accurate assessment of the toxic health risk.

Drugs abuse is on the rise in our society and with the growing use of methadone in adults, the number of poison exposure cases in infants is increasing. Drug-related deaths in infants is more difficult to identify due to the different pharmacological parameters and the lack of specific data. Moreover, it is difficult to distinguish between systemic incorporation into hair after ingestion and external contamination. However, the difference is crucial to assess particularly dangerous situations and evaluate the health risk. Therefore, the parameter of hair concentrations should not be used alone since it could be difficult to interpret. As a consequence, forensic experts should take into consideration this datum together with a thorough investigative history and laboratory and autopsy findings.

Pediatric exposure to drugs is relatively rare but concerning, with potentially serious complications, such as seizures, dysrhythmias, and death. The variations in pediatric metabolism and the lack of neonatal data make a precise diagnosis difficult. This may contribute to these cases being labeled as “undetermined” by the forensic community. Hair has been suggested to be a suitable matrix to document repetitive exposure to drugs.

Reported here is the case of a sudden and unexpected death of a 5-month-old girl born to drug-addicted parents. The infant was delivered by cesarean section after a full-term pregnancy. Newborn urine was positive for methadone, as her mother had a known history of methadone intake during pregnancy. The baby had a withdrawal syndrome treated by phenobarbital. The infant was discharged from the hospital healthy. At home, she was both breast- and bottle-fed. Four months after birth, the baby had a seizure, spontaneously solved, and Electrocardiogram (ECG) and Electroencephalograph (EEG) were unremarkable. The day the child died, she had fever for which she had been given acetaminophen. In the evening, she had her meal and fell asleep, but soon after she started to vomit. Emergency services were called, and cardiopulmonary resuscitative maneuvers were initiated by the parents. The infant arrived at the emergency room in asystolic arrest and could not be resuscitated. Blood tests showed anemia and leukocytosis. An autopsy was disposed by the local prosecutor.

At the external examination, traumatic injury was not observed; postmortem Computed Tomography (CT), autopsy, and microbiological and histopathological examination revealed no significant findings. Also, the toxicological analyses, Liquid Chromatography/High Resolution Mass Spectrometry (LC/HRMS), performed on the peripheral blood and organ specimens were negative. Instead, hair analysis showed a strong positivity for methadone (2.3ng/mg) and its metabolite EDDP (0.1ng/mg), cocaine (2.6ng/mg) and its metabolite benzoylecgonine (0.75ng/mg), 6-monoacetylmorphine (a direct metabolite of heroin, (0.2ng/mg), and morphine (1.0ng/mg), suggesting chronic exposure to cocaine, methadone, and heroin. Literature reveals the association between sudden death (due to arrhythmias, seizures, or respiratory failure) and chronic exposure to such substances; accordingly, the infant’s death can be reasonably attributable to the combined effect of all these drugs.

In young children, the major problem in testing hair for drugs is the interpretation of the findings. Hair is thinner and more porous than in adults and therefore the risk of contamination by sweat is higher, the growing is asynchronous, and it is difficult to detect. Transfer through sweat can be the result of contact with pillows, bedding, parental kisses, and skin contact, and it is the first reason to have positive hair tests in children. Considering that metabolites could also be present in large amounts in sweat, their presence cannot be discriminative for drug administration or accidental intake. Moreover, drugs positivity in children’s hair could be the result of *in utero* exposure.

#### Drug Intoxication, Sudden Infant Deaths, Hair Analysis



### E29 A Novel Vapor Delivery Device for Homemade Explosive Analysis

*Laurn DeGreeff, PhD\*, U.S. Naval Research Laboratory, Washington, DC 20002; Christopher J. Katilie, Nova Research, Inc., Alexandria, VA 22308; Susan Rose-Pehrsson, PhD, U.S. Naval Research Laboratory, Washington, DC; Michael Malito, Nova Research, Inc, Alexandria, VA*

**Learning Overview:** After attending this presentation, attendees will have learned about binary explosive mixtures used in Homemade Explosives (HMEs) and will understand safety concerns that limit work, be it research, vapor generation, or canine training, with such materials.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a method of HME vapor delivery and generation using two novel odor delivery devices and how such devices can be used to overcome the challenge of working with binary explosive materials.

HMEs are commonly found in Improvised Explosive Devices (IEDs) that have become increasingly commonplace in Middle East conflicts and also pose a threat at home. HMEs are often composed of simple, binary mixtures of an oxidizer and a fuel. The individual components often have common, innocuous uses, independent when separated, but form an explosive mixture when combined. Common examples of oxidizers are Ammonium Nitrate (AN) or Potassium Chlorate ( $\text{KClO}_4$ ), and examples of fuels include, but are not limited to, aluminum powder and fuel oil.

Mixed explosives are often difficult and expensive to safely obtain, store, and transport. Safety measures may limit use to same-day production with strict mass limits and disposal protocols. These regulations often restrict or preclude training, analytical research, and other testing using the mixtures themselves. For this reason, detection protocols often focus on the detection or sensing of the oxidizer alone. Canines, for example, are often trained on solely AN and not AN-fuel mixtures, though recent canine evaluations have shown that the canines perform better when trained to the mixed components.

A novel Mixed Odor Delivery Device (MODD) was designed to safely contain the solid or liquid components of HMEs and deliver the HME vapor signature for passive or active sampling. Within the device, vapors from the separately housed components mix as they move through the device toward the outlet. The resulting mixed vapor is representative of that which would be achieved from the actual mixed explosive material. For active sampling, air flows from an external source, through the device, carrying the mixed analyte vapor toward the instrument of choice. For passive sampling, component vapor diffuses from the bulk material through a Teflon<sup>®</sup> neck, where vapors mix before exiting the device at a bowl-shaped outlet. Both active and passive transport devices have been tested with surrogate components as well as actual explosive components. The MODD offers transportability and ruggedness for field use with minimal sample size requirement and is easily adaptable for the varied components one might encounter in the field.

Initial characterization of the active MODD was conducted using single compounds characteristic of HME components. The equilibration time for each compound in the device and the stability of the equilibrium concentration over time was measured with a focus on flow rate and temperature dependence. Run-to-run and day-to-day reproducibility in vapor generation using the MODD was illustrated by the generation of octane and 2-phenylethyl alcohol (separately) from the MODD. The equilibrium vapor concentrations for both compounds were shown to be stable for more than four hours and were reproducible over several days, respectively.

A computational model was used to predict the transport of analyte vapor by diffusion within the passive MODD. The focus of the analysis was the symmetry of the analyte vapor concentration across the MODD outlet. Assuming a symmetric concentration field at the outlet, the vapor profile would be equal whether approached from the left or from the right, and the components in the MODD would not be recognizable as separated, but instead only as a mixed vapor. The models demonstrated that the concentration field becomes symmetric due to the small central channel separating the upper and lower chambers. Laboratory evaluations were completed to ensure the model was satisfactory and that the MODD delivers a uniform mixture of vapor at a detectable level. Based on this data, it can be shown that the MODD accurately portrays the mixed vapor of these separated compounds.

#### Vapor Detection, Homemade Explosives, Vapor Delivery





### E30 The Current State of Homemade Explosive Detection by Canines—Research and Knowledge Gaps

Lauryl DeGreeff, PhD\*, U.S. Naval Research Laboratory, Washington, DC 20002; Kimberly Peranich, BS, NSWC IHEODTD, Indian Head, MD 20640; Alison G. Simon, PhD, Washington, DC 20375

**Learning Overview:** After attending this presentation, attendees will have learned of the changing trends in explosive threats and how this pertains to canine detection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing attendees with an understanding of where there are knowledge gaps in regard to canine detection of explosives, why these gaps exist, and how they may be filled.

The gold standard of non-contact explosives detection in real-time is canine detection. Canines are sensitive and selective detectors that can easily be trained to locate novel materials. However, there is often little research to support canine training and deployment. Per this study's research, the most recent review of the scientific foundation for canine research was published in May 2001, prior to the September 11, 2001, attacks and the Boston Marathon bombings of 2013.<sup>1</sup> Both of these events broadly altered law enforcement, affecting every aspect of training and deployment, including the canine sector.<sup>2,3</sup> Compounding the issue, Homemade Explosives (HMEs) have emerged over the last two decades as the predominate weapon used against the United States, but there are still significant gaps in the literature and research on this topic. HMEs are easily constructed or synthesized using commercial ingredients and equipment that are more difficult for traditional methods to detect. Even with the diversity and power of the canine detector, there are minimal resources available for canine research, financial and otherwise. This leads to a dearth of understanding of this detector, which ultimately has a negative impact on canine detection proficiency.

A thorough survey was conducted to assess current and past research in the field of canine detection. This evaluation included research on the detection of explosives, as well as other targets relevant to military and law enforcement. This includes narcotics, human remains, and human tracking, as well as associated research in the fields of veterinary, neuro, and behavioral sciences as they relate to canine detection. Information was collected in the form of surveys provided to researchers in the field of canine detection across many disciplines, as well as to operational users, including canine handlers and trainers. In addition, an extensive review of both peer-reviewed literature and government reports was conducted and a searchable database of canine detection-related literature was compiled. Data from both the surveys and the literature review were used to identify critical knowledge gaps and user needs. Suggestions for appropriate topics for future funding to best serve the canine detection community, with the goal of improving the canine as a versatile field-detector, have been made. Areas of recommended future research include the following: (1) odor delivery, diffusion, and availability; (2) training aids, including storage and shelf life; (3) HMEs; and (4) veterinary health and canine ability/proficiency. It was also suggested that a repository for canine research be created for users and future researchers and maintained on a regular basis. While the body of canine research continues to grow, there remains a need to conduct further research to ensure the ability of these detectors to meet the changing nature of warfare.

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#### Canine Detection, Homemade Explosives, Explosives Detection



### E31 The Detection of Risk Factors of Elder Abuse in Domestic Settings

Maria Carla Mazzotti, MD\*, Department of Medical and Surgical Sciences, Unit, Bologna, ITALY; Alberto Amadasi, MD, Università di Bologna, Bologna 40126, ITALY; Anna Maria Govi, MD, Department of Medical and Surgical Sciences, Bologna 40126, ITALY; Giancarlo Salsi, PhD, Department of Medical and Surgical Sciences, Bologna 40216, ITALY; Susi Pelotti, MD, University of Bologna, Bologna 40100, ITALY

**Learning Overview:** After attending this presentation, attendees will understand: (1) some principles of elder abuse, (2) the different practice settings and various forms in which it may present, (3) the current prevalence, and (4) that available data still represents an underestimation of the phenomenon, as the abuse is frequently unreported.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a key aspect in the detection of risk factors of elder abuse in domestic settings, increase awareness of the existence of this particular form of abuse, and guide the training of health care providers for primary and secondary prevention. Indeed, the results of this study provide a framework for forensic researchers and practitioners on a phenomenon that is still submerged, unlike other forms of domestic abuse.

In Italy, limited research has been conducted on indicators of individuals who may be more susceptible both to perpetrate violence or to be susceptible to abuse. The purpose of this study was to investigate the presence of potential forms of elder abuse in domestic settings by analyzing risk factors related to both the caregiver and the elderly person in order to assess individuals who may be at risk for, or victims of, abuse.<sup>1</sup>

Data was collected on a sample of 88 volunteers of an Italian Association named “Auser,” each of whom was in charge of an elderly person assisted by a caregiver in a domestic setting. A previously designed and validated questionnaire (Indicator of Abuse (IOA)–Screen) was used to collect data from participants.<sup>2</sup> The questionnaire consisted of 13 questions for the caregiver and 24 questions for the elderly person. Multiple logistic regression models were used for data analysis in Stata/SE 10.1 for Microsoft® Windows.

A situation of possible or suspected abuse was detected in 59% of the cases, of which 69% identified the caregiver as a family member. The average age of the caregiver was 55.5±16.3 years, while the average age of the elder was 80.4±8.2 years. The most frequent risk factor for elders to be exposed to higher rates of abuse were dementia, followed by financial dependence. Concerning the caregiver, alcoholism was the most common risk factor. Logistic regression did not show a difference in gender (both in caregivers or elderly people) as statistically significant.

In view of the rapidly aging population, the detection of elder mistreatment is emerging as a public health priority.<sup>3</sup> Indeed, as the population ages, the percentage of older adults experiencing abuse, neglect, or exploitation is expected to grow. In such scenarios, interventions to prevent or reduce elder abuse are crucial. This study has found potential risk factors of caregivers and elderly people that may be preventable, to an extent. It is therefore recommended that medicolegal experts become familiar with elder abuse and its potential risk factors.

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#### Elder Abuse, Risk Factors, Prevention



## E32 Forensic Evidence and Investigation Implications in Domestic Violence Incidents Involving Non-Fatal Strangulation

Katherine M. Brown, PhD\*, Tarleton State University, Bryan, TX 77807; Tara O. Shelley, PhD, Tarleton State University, Fort Worth, TX 76116; Lacy Hensley, MA, One Safe Place, Fort Worth, TX 76104

**Learning Overview:** After attending this presentation, attendees will: (1) understand the nature and extent of domestic violence incidents involving Non-Fatal Strangulation (NFS); (2) be able to recognize the physical symptoms and injuries associated with NFS; (3) be aware of the forensic evidence and investigative implications of these incidents; and (4) be able to identify additional correlates and risks associated with NFS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing competence in response to NFS domestic violence incidents. This presentation will increase recognition of physical signs and symptoms and other important forensic evidence and will investigate implications in these incidents. Specifically, this presentation will allow attendees to identify evidence and additional correlates and risks associated with NFS in domestic violence incidents and will also give first responders and investigators information about an underresearched area.

It is estimated that the number of women who experience NFS is at least 10% and could be as high as 68% for female victims of Domestic Violence (DV).<sup>1-4</sup> It is also estimated that between 22%-35% of women who visit the emergency room are there for medical problems related to DV, and one out of every three female trauma patients is a DV victim.<sup>5</sup> Unfortunately, most victims of NFS will not seek medical treatment and law enforcement frequently misses, or fails to properly document, the external evidence, signs, and symptoms of strangulation.<sup>2-6-9</sup> While some research indicates that 40% of DV victims were strangled, only about 10% actually reported to law enforcement and emergency personnel that they had been strangled and were experiencing physical symptoms.<sup>10,11</sup> Increasing their risk, victims of DV who experience NFS are *seven times more likely* to be victims of attempted homicide and *eight times more likely* to subsequently become victims of a homicide.<sup>1,6,7,12-14</sup> The children of victims are also at risk. The presence of children to NFS in DV incidents also places them at risk of adverse health effects, and estimates show that between one and ten children are at risk because of exposure to DV.<sup>15</sup> In addition to adverse impacts on the child, the presence of a child in a DV incident may increase the strangulation risk to the victim. In approximately half of strangulation cases, children were present at the time the victim was strangled.<sup>8</sup>

This presentation examines NFS DV incidents involving victims seeking services from One Safe Place Family Justice Center (OSP) in Fort Worth, TX, from January 1, 2016, to June 30, 2017 ( $n=1862$ ). Researchers collected and analyzed OSP client records from numerous sources: (1) intake and incident forms (e.g., previous victimization, children, relationship characteristics, nature of abuse); (2) a danger assessment instrument (e.g., risk factors associated with homicide in violent relationships); and (3) a strangulation questionnaire administered to clients reporting strangulation in the danger assessment instrument (e.g., frequency of strangulation, symptoms, and injuries).

The findings include descriptive data regarding the nature and extent of strangulation physical symptoms and other factors that provide important forensic and investigative implications. Researchers also utilized logistic regression to determine correlates of NFS. Preliminary results indicate that 56% of the sampler had been strangled ( $n = 663$ ), 73% of these victims reported they had a child under the age of 18, and 48% indicated they had children in the household who were not the offender's biological child. Victims also reported an array of physical strangulation symptoms: difficulty in breathing (68%); lightheaded or dizziness (54%); vision or hearing issues (27%); loss of consciousness (53%); and some reported the loss of bodily functions (12 reported urination and 2 reported defecation). Post-strangulation incident, victims reported: bruising (56%); difficulty swallowing (42%); difficulty breathing (33%); and changes in their voice (35%). Only 5.5% of strangulation cases had no law enforcement involvement, and 74% of victims did not seek medical treatment for their injuries.

When cross-referencing danger assessment scores with the occurrence of strangulation, it was discovered that 72% of victims in the extreme danger range had been strangled (versus 28% that had not been strangled). The difference between the two groups was statistically significant. Logistic regression results indicate that victims are also *significantly more likely to experience strangulation* if: their abuser has previously threatened to kill them; if the abuser has previously avoided arrest; if children present were not related to the abuser; if verbal and physical abuse had previously occurred in front of children; and if the offender had beaten the victim while she was pregnant. In addition, the practical implications for evidence collection and investigation in NFS incidents will be presented.

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### **Non-Fatal Strangulation, Forensic Evidence, Investigation**



**E33 Staging: A Human Rights Issue**

*Edwin O. Olaya Molina, BA\*, Bogotá 111321, COLOMBIA; Daniela Tafur\*, Fiscalía General De La Nación, Bogota, Cundinamarca, COLOMBIA*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### E34 Forensic Investigation and Criminal Profiling of Very Unusual Infanticide in Italy

*Luciano Garofano, PhD\*, President of Accademia Italiana di Scienze Forensi, Parma 43100, ITALY; Laura Volpini, PhD\*, Rome 00174, ITALY*

**Learning Overview:** After attending this presentation, attendees will understand the dynamics of a very specific infanticide that took place in Italy in 2002, in which a mother killed her baby. There will be discussion of the analysis done of Bloodstain Pattern Analysis (BPA), the crime scene analysis used to solve the murder, and the method adopted for the analysis of this crime.

**Impact on the Forensic Science Community:** This presentation will impact the forensic scientific community by discussing the importance of a multidisciplinary approach and how it could be effective in the resolution of complex forensic cases.

The case concerns an infanticide which occurred in Cogne, a small mountain village in northern Italy in January 2002. The case had a great media echo that lasted for years due to the duration of the investigation and the trial, both on television and in the press.

The body of a 3-year-old baby was found in the parents' bedroom, completely smeared with blood. Many technical activities were conducted by the specialists of the forensic science lab of the Carabinieri-Parma. A thorough examination of the crime scene and further analyses of hundreds of pieces of forensic evidence were subsequently conducted at the urging of the defense experts. The total lack of fingerprints or DNA other than the victim's family, as well as the particular distribution of bloodstains, progressively focused suspicion on the mother, even though she proclaimed herself innocent.

Great attention was dedicated to the physical characteristics and distribution of bloodstains, which were analyzed through a rigorous application of BPA. To do this, the murder room was reconstructed in order to test two main hypotheses: (1) an attack carried out by an aggressor positioning himself exclusively next to the bed; (2) an attack carried out by an aggressor who—wearing the mother's pajamas—initially hit the victim beside the bed and then moved on the bed, hitting the victim frontally and inflicting wounds only on his head.

A forensic psychiatric assessment on the woman was also performed, as well as a second psychiatric assessment ordered by the Court during the second-degree trial. The psychiatric examination conducted during the appeal trial used an innovative methodology in the forensic field. The various tasks were distributed among a team of experts, then the results of the work were reassembled to outline the psychological framework and the criminodynamics of aggression in an integrated manner. After a close confrontation between all the experts, including the results achieved by the psychiatric examination, the only sustainable hypothesis was the criminal responsibility of the mother. After three degrees of judgment, she was sentenced to 16 years imprisonment.

The oral presentation of this case will reconstruct with great detail the criminal dynamics, the psychological motivations at the origin of the crime, and the psychological profile of the mother. Salient data referable to infanticide in Italy will also be discussed.

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#### Forensic Investigation, Criminal Profiling, Infanticide



### E35 Crime Scene Reconstruction in an Organization of Scientific Area Committees (OSAC) World

*Peter R. Valentin, MSFS\*, University of New Haven, West Haven, CT 06516*

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**Learning Overview:** After attending this presentation, attendees will understand the important role crime scene reconstruction has in the understanding and interpreting of forensic evidence in both criminal and civil cases. The conspicuous absence of a separate crime scene reconstruction subcommittee as part of the National Institute of Standards and Technology (NIST) Organization of Scientific Area Committees (OSAC) tacitly suggests that the interpretation of the physical evidence is best handled in a courtroom or otherwise as part of the legal process.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by starting a conversation about what happens to the evidence we analyze. Specifically, should we develop and apply scientific standards to how we interpret evidence in much the same way we apply standards to how we examine evidence?

With the creation of the OSAC under NIST, considerable progress has been made to strengthen the scientific foundations and processes in many forensic disciplines. What is lacking is the development of a similar process for crime scene reconstruction. When the significance or interpretation of forensic evidence is disputed, and in particular when that information needs to be integrated into the body of investigative information, a crime scene reconstructionist can help evaluate possible scenarios. With so much emphasis on solidifying forensic science, why has the interpretation of that information been left to be decided in a courtroom? Shouldn't the interpretation of forensic science (in the context of a case) be subject to the same types of guidance we seek to codify as part of the OSAC standards development process?

The absence of a reconstruction committee suggests that either the forensic science community does not concern itself with the interpretation of its work product or that we believe that this is adequately handled as part of the adversarial court system. Neither is (or should be) true. While an OSAC crime scene investigation subcommittee already exists, the sheer amount of work before them in addition to reconstruction makes this too large a task for a single subcommittee to handle. Additionally, with all the potential disciplines reconstruction can draw their information from, a dedicated committee with representation from many disciplines should be developed.

At the root of many legal cautionary tales on the use of forensic science are issues of interpretation and presentation of forensic evidence. If we fail to act decisively, we run the risk of having this critical function curtailed. Shouldn't we provide guidance for the reasonable interpretation of forensic evidence just as we do for understanding the results in a specific discipline?

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#### Crime Scene Reconstruction, OSAC, Standards



### E36 Dermoscopy in Forensic Medicine

*Benjamin Mokdad\**, Rouen, Normandie 76000, FRANCE; *Anne-Claire Lhoumeau, MD, CHU Charles Nicolle, Institut Médico-Légal, Rouen 76031, FRANCE; Pauline Jorda, Rouen, Seine Maritime, FRANCE; Paul Young, MD, Chu Rouen, Rouen 76000, FRANCE; Gilles P. Tournel, Rouen, Seine Maritime 76000, FRANCE*

**Learning Overview:** The goal of this presentation is to demonstrate that dermoscopy is a simple, fast, and cheap technique useful in legal medicine that can substitute for DNA identification techniques in many situations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a new technique for the identification and examination of stab wounds and gunshot wounds.

Dermoscopy is a technique initially used for the early detection and diagnosis of melanomas. As a useful tool, it was further developed to diagnosis other skin lesions that include skin cancers and inflammatory and infectious diseases. Per research, this work reports on the first use of dermoscopy in forensic medicine. For one year, the Forensic Medicine Department at the University Hospital of Rouen in France has been equipped with an immersion dermoscope (Heine® 20). It has been used systematically in many medicolegal situations. Three cases utilizing dermoscopy in forensic medicine will be discussed.

**Fingerprints:** The formal identification of bodies by the fingerprint method is an empirical method that has lost its beauty since the discovery and use of DNA. However, fingerprint analysis by dermoscopy coupled with photography allows a clear image of dermatoglyphs, even when there is no longer an epidermis or in cases of mummified skin. On very damaged bodies for which the traditional method of fingerprints (powders) is no longer possible, the dermoscope provides images of sufficient quality for identification. In addition, it is possible to observe the sweat pores, which are not currently a point of comparison, despite a recent study of interest in identification. Considering sweat pores, a square millimeter fingerprint fragment has tens to several hundreds of points for comparison. This is enough to certify an identity and to propose a new fingerprint analysis method. Dermoscopy allows high-quality fingerprints that are directly scanned and therefore easily comparable to a national fingerprint database.

**Gun wounds:** Dermoscopy directly visualizes the soot and grease traces at wound sites and makes it possible to quickly distinguish between the inlet and the outlet wounds without having to resort to a pathological examination. This is accomplished by using the dermoscope to identify grains of powder and other trace residues at the wound.

**Stab wounds:** Dermoscopy allows for a fast and precise analysis of stab wounds. It allows a more rigorous analysis of the ends and edges of wounds than is possible solely with a visual examination. This makes it possible to discern if the knife used possessed a single or double edge. The edges of the wound can adopt a bifid or unique wound characteristic that is easily analyzed by dermoscopy and can guide both the type of blade and the possible blade movements.

Several techniques are recognized and validated to obtain formal recognition of deceased subjects. In the case of very damaged bodies, the examination of the dentition is often not very conclusive. The realization of fingerprints is a well-established technique, but difficult or impossible to use in cases of hand alteration (drowning, mummification). In these cases, performing a DNA search is a destructive technique because it requires the removal of sufficient muscle (often psoas, as it is better preserved) or bone marrow (whole femur). In addition, it is a more complicated and expensive technique, requiring extra steps (lysis, elimination of proteins and acids, concentration of DNA, etc.). Dermoscopy allows visualization of the sweat pore. Thus, it makes it possible to obtain a much greater number of comparison points than with a conventional technique. Sweat pores have already been identified as a reliable element for identification. This technique allows a considerable reduction of the risk of misidentification because a multiple fold number of comparison points are available on a small surface (1cm<sup>2</sup>). The comparison of the sweat pores is not currently used in France but is already in use by some foreign police departments.

### Dermoscopy, Fingerprints, Forensic Medicine





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### E37 Diligence, Dedication, and Devotion: Reaching Back Into History for the Image of a Victim

*Sandra R. Enslow, BA\*, Los Angeles County Sheriff's Department, Monterey Park, CA 91755*

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**Learning Overview:** After attending this presentation, attendees will understand some of the difficulties that forensic artists often encounter with facial reconstructions. Facial reconstructions are developed from the skull or, in this case, photographs of the skull in order to get a better understanding of what a victim looks like. This information may possibly identify a victim, even if the case is 28 years old.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the work that forensic artists do for their detectives and investigators. The majority of this work goes beyond drawing a composite of a suspect. In this presentation, an outstanding cold case criminal investigation that requested a facial reconstruction will be presented. The female victim's skeletal remains were discovered in 1980 in a dry riverbed in a suburb of Los Angeles County. The skull had a bullet hole in it. This informative presentation will highlight original photographs, step phases, and finalized images of the victim's face.

The skeletal remains were cremated at some point, which sometimes happens. This does not deter a forensic artist, but rather sends them down other avenues for the information they seek. Vital information and clues about the face can be found in the anthropologist's final report. This turned out to be the case here. The Los Angeles County Coroner's Office anthropologist in 1980 noted descriptive features about the victim's palate and orbital sockets. This information was extremely helpful and gave the forensic artist specific direction in producing those parts of the image.

There were two challenges in this case. First, without the actual skeletal remains, the photographs of those remains became the crucial source for reference. The photo of the skull, while somewhat clear, was taken for general documentation purposes only. This photo was not to the standard used for a facial reconstruction. The care and specificity that a forensic artist would have applied to the angle and presentation of the skull for photography purposes was not there. Approximating the Horizontal Frankfort position was not possible. There were no lateral views of the skull.

The second challenge was the missing mandible. Many skulls are discovered without this important part of the skull. For forensic artists, this just goes with the territory and is often the rule rather than the exception. In Photoshop®, a new mandible is generated and applied in the photographic phase. This allows the continuation of the facial reconstruction, although it is not ideal.

The skull needed to be reconstructed before the facial reconstruction could even begin. The skull's right side was intact, but the zygomatic bone on the left side had been broken off. This had to be approximated and accepted as similar to the right side.

This case was considered to have possible connections to another serial murder series in the Southern California region. Whether this individual had been a victim of the serial murderer remains to be seen, although the location and time frame position her as a candidate. Creating the image, then obtaining an identification of the victim were the first two steps in the process of finding a possible connection. Much of that casework is still ongoing.

Forensic art is a rapidly expanding discipline that requires specialized training, above and beyond advanced drawing skills. The forensic artist is called upon for many different skill sets that assist in the identification process. Applications relate closely with those of other disciplines of the forensic sciences, especially forensic anthropology, forensic odontology, and forensic psychology.

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#### Facial Reconstructions, Forensic Art, Forensic Imaging



**E38 How Accurate Is Forensic Image Identification of Surgically Altered Faces?**

*Petra Urbanová, PhD\*, Masaryk University, Brno, Czech Republic 611 37, CZECH REPUBLIC; Hana Eliasova, PhD, Prague 170 89, CZECH REPUBLIC; Tatjana Dostalova, MD, PhD, Motol University Hospital, Prague 150 06, CZECH REPUBLIC*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### **E39 Forensic Validity and Usefulness of the Apache Tracking Method: A Multifaceted Research and Development Protocol**

*Joseph L. Lewis III, MFS\*, National University, Forensic Sciences Program, La Jolla, CA 92037; Ismail M. Sebetan, MD, PhD\*, National University, La Jolla, CA 92037-1011; Paul Stein, PhD\*, National University, La Jolla, CA 92037*

**Learning Overview:** After attending this presentation, attendees will develop an understanding of the Apache Tracking Method and its forensic relevance and validity regarding how speed of human travel can be determined from shoe impression details left in substrates affected by the kinetic energy transference associated with forward movement. Average speeds of travel can be determined from the track details denoting forward motion of subjects.

**Impact on the Forensic Science Community:** This presentation will impact attendees by adding another novel level of scientific knowledge for biometric identification and investigation. This is noteworthy since there has not been adequate empirical data collected about tracking methods. This would offer useful information about suspect identification, crime scene reconstruction, pursuit of criminals, and apprehension of fugitives.

The key objective of this study was to determine if travel speeds can be approximated (mph) by interpreting the kinetic energy transfer details left in the shoe print impressions in a given substrate. If correct, then there should be no differences observed in the speed assessment of three subjects traveling at similar speeds. The speed assessments would be criteria dependent based on the shoe impressions, not subject dependent, assuming roughly similar weights. For this study, three male volunteers (average weight: 173.2 lbs, +/-19.4lbs SD) were recruited to travel through a tracking box (3.5 ft. wide, 12 ft. long, and 4 in. deep) filled with damp sand (Zero Earth) and with travel speed ranges of 6–8mph, 9–11mph, and 12–14mph measured by Doppler radar. This experimental “field” model was done to determine what correlation (if any) the subjects and travel speeds may have relative to pressure release scales (The Wave, The Double Wave, The Disk, The Disk-Fissure, The Disk-Crumble, The Dish, The Dish-Fissure, The Dish-Crumble, and The Explode-Off). The shoe prints left in the Zero Earth substrate were photographed and analyzed using the forward motion pressure release analysis and categorization method.<sup>1</sup> Any statistical difference across the test subjects at the travel speed ranges and pressure release categorizations were analyzed by chi-squared ( $\chi^2$ ) test (p value <0.05).

There was no significant difference between the three subjects at the three travel speed ranges. The three speed ranges can be differentiated by pressure response in the Zero Earth substrate, across nine different pressure response scales. The same classes of kinetic energy transfer details were detected at the same speed ranges. This allowed for a reliable estimate of subject speeds by shoe print analysis. The chi-squared test indicated there was no difference in the likelihood of making the same class of substrate pressure details (pressure releases) for the respective travel speed ranges.

The conclusion of this presentation is that specific travel speeds can be approximated by reading the forward motion details (pressure releases) in the shoeprint substrate. It was determined that The Disk pressure release class was found in all three subjects’ shoe print series at the 6–8mph travel speed range, The Disk-Crumble pressure release class was found in all three subjects’ shoe print series at the 9–11mph travel speed range, and The Explode-Off pressure release class was found in all three subjects’ shoe print series at the 12–14mph travel speed range.

#### **Reference(s):**

<sup>1</sup>. Brown, T. *The Science and Art of Tracking*. New York, NY: The Berkley Publishing Group. 1999.

#### **Forensic Sciences, Biometrics, Apache Tracking Method**



**E40 A Holistic Assessment of Job Satisfaction of Forensic Psychology Professionals**

*Dione Washington\*, Beltsville, MD 20705*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### **E41 A Forensic Criminological Approach to Oil Spill Investigation**

*Edidiong Mendie\*, Houston, TX 77007; Ashraf Mozayani, PharmD, PhD, Texas Southern University, Houston, TX 77004*

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**Learning Overview:** After attending this presentation, attendees will better understand the correlation between forensic science procedures and their impact on oil spill identification. This awareness will help attendees be more familiar with the role forensic science plays in oil spill cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing understanding of why the evidence management procedures should be given equal relevance in oil spill retrieval to avoid contamination. This presentation will also provide recommendations as to the need for adopting a benchmark that will be employed by forensic analysts that use forensic techniques in identifying oil spills.

Oil spill disasters have become a global problem that adversely affects people and the environment. When there is an oil spill, forensic experts are involved because the evidence retrieved from the crime scenes are sent to the forensic laboratory for analysis. Flowing from this, environmental forensics has emerged as a tool for regulating environmental contamination, especially those arising from oil spills. This has been achieved through different oil spill identification mechanisms. Oil spill identification involves techniques employed to discern causes of oil spills. Thus, forensic techniques such as Gas Chromatography/Mass Spectrometry (GC/MS), and Polycyclic Aromatic Hydrocarbons (PAHs) play a significant role in determining the source of spilled oil so that we are better able to deduce their occurrences.

Earlier studies have researched various forensic methods used in oil spill identification. This was achieved by retrieving data from oil spill locations, comparing the chemical components from such investigation sites with substances from other locations, and analyzing the results to determine whether or not a match exists. However, questions as to whether these forensic studies were conducted following laid-down standards have been left unanswered.

In understanding the oil spill identification process, this report employed a case study approach. It divided the analysis into three stages, namely the pre-analytical, analytical, and post-analytical phases. The pre-analytical stage assessed the retrieval of evidence from oil spill sites. It examined whether evidence retrieved from select oil spill sites were conducted using forensic techniques. The analytical phase focused on evidence collection and its perceived impact on the oil spill process to avoid contamination. It emphasized the compliance steps required for the purpose of evidence handling. The post-analytical phase studied the overall effect on the criminal justice system, such as the courts, that relies on such evidence and the testimonies of expert analysts.

This exhibition highlighted the need to properly collect and preserve evidence from the oil spill site that will be sent to the laboratory for testing. The oil spill investigation scene ought to be treated as a crime scene investigation to prevent the loss of important evidence. Thus, retrieval of evidence from oil spill sites must be performed by following all relevant evidence management procedures.

Being the first of its kind, the purpose of this analysis is to offer the recommendation that a universal framework be adopted in forensic testing of oil spill cases. By doing so, this report seeks to assure the public of the credibility in the forensic science discipline and its procedures.

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**Oil Spill, Forensic Technique, Criminal Justice**



### E42 Validation of a Dry Vacuum Swabbing Method for the Recovery of Epithelial Cells From Handled Porous Substrates

*Yih Ling Saw, MSFS\*, Center for Forensic Science Research & Education, Willow Grove, PA 19090; Kevin J. Piccirilli, MSFS, Utah Bureau of Forensic Services, Taylorsville, UT 84129; Sara G. Goodman, MSFS, Columbia, SC 29210; Heather E. McKiernan, MSFS, Center for Forensic Science Research & Education, Willow Grove, PA 19090*

**Learning Overview:** After attending this presentation, attendees will understand the value of employing an efficient collection technique for the analysis of touch-based samples and be able to evaluate the application of dry vacuum swabbing on commonly handled porous evidentiary materials.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing validation data supporting the use of an alternative collection technique, a dry vacuum swabbing method, for handled porous items of evidence for genetic analysis. Evaluation of the efficiency, reliability, and potential limitations of this collection methodology will help analysts decide under what conditions it could successfully be applied to forensic casework.

Analysis of low template touch DNA samples has always been a challenge for forensic analysts. Over the years, the field of forensic science has seen numerous improvements in technology in terms of analytical approaches and reaction chemistries that have been made commercially available to improve the quantitative and qualitative recovery of such samples. However, in order to increase the initial yield of recovered DNA, optimization of the collection technique applied is just as crucial to the DNA workflow.

Several collection techniques have traditionally been employed in the collection of touch-based samples in crime laboratories. More recently, a commercially available wet vacuum collection application has also been applied to casework samples. Each of these techniques have their own benefits and challenges, some working better on more non-porous substrates, others working better over larger surface areas, and some showing poor recovery of epithelial cells.

Several mock items of evidence were handled, then samples were collected using several collection techniques: double swabbing, scraping, tape lifting, and the in-house dry vacuum swabbing technique. Substrates evaluated in this study included an array of commonly encountered porous substrates submitted for touch DNA analysis, such as articles of clothing and rope. In one scenario, individuals wearing long-sleeved cotton shirts were dragged across the floor by their arms. Each of the collection techniques were applied in an attempt to target the “suspect” versus the “victim” DNA. Statistically significant increases in the amount of recovered quantifiable DNA were observed when using the dry vacuum swabbing technique ( $F_s=8.270$ ;  $df=3,28$ ;  $p=0.0004$ ) when compared to the other collection techniques evaluated. This subsequently resulted in the generation of robust, high-quality Short Tandem Repeat (STR) profiles in which 100% of the “suspect’s” alleles were detected. In contrast, traditional collection techniques produced lower quality STR profiles with a high degree of allelic dropout. In another scenario, “victim’s” hands were tied together by the “suspect’s” using natural fiber rope. Once again, all collection techniques were utilized and evaluated. Samples from the middle of the rope (the portion in contact with the “victims”) were collected separately from the ends of the rope (those in greater contact with the “suspects”). Once again, downstream genetic analysis showed that the dry vacuum swabbing collection technique recovered the highest quantity of DNA from the rope ends when compared to all other collection techniques assessed, recovering 80+% of victim and perpetrator profiles from both the center and ends of the rope, respectively. Recovery of low-level touch DNA from simulated sexual assault scenarios in which “suspects” forcibly removed “victim’s” denim jeans and bra also showed drastic differences between the performance of the assessed techniques, with the dry vacuum swabbing collection technique recovering an average of 3.81% more male DNA attributable to the “suspect” than the other collection techniques.

As the number of touch type DNA samples being submitted for casework analysis continues to rise, it is imperative that front-end optimization is achieved to bolster the success of downstream analyses. While recovery of touch DNA has historically been challenging for forensic analysts, this research has proved that the dry vacuum swabbing collection technique may be an ideal workflow solution for the efficient recovery of epithelial cells from porous substrates, especially in instances in which targeted collection is needed to avoid wearer or “victim” DNA. Additionally, this technique is easy and cheap to assemble in a forensic laboratory and does not require the purchase of dedicated and expensive commercial products.

#### Touch DNA, Dry Vacuum Swabbing, DNA Collection



**E43 Investigating Touch DNA Recovery From Ivory as a Method to Identify Poachers and Traffickers**

*Emily L. Horrocks, MSc\*, University of Portsmouth, Portsmouth, Hampshire PO1 2QQ, UNITED KINGDOM*

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**THIS ABSTRACT WAS NOT PRESENTED.**



## E44 Validation of the PrepFiler® Express BTA™ Forensic DNA Extraction Kit

*Jessica Slater\**, Huntington, WV; *Dorothy M. Catella*, MSFS, Oakland County Sheriff's Office, Pontiac, MI 48341; *Megan Connolly*, MPS, Oakland County Sheriff's Office, Pontiac, MI 48341; *Kelly Beatty*, MSFS, Marshall University Forensic Science Center, Huntington, WV 25701

**Learning Overview:** After attending this presentation, attendees will have information concerning the use of PrepFiler® Express BTA™ and the possible substrates for extraction with PrepFiler® Express BTA™, as well as the advantages and disadvantages of using PrepFiler® Express BTA™.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community, especially those in the DNA biology analysis field, by describing a method of obtaining a DNA Short Tandem Repeat (STR) profile from substrates with a limited quantity of DNA by using the PrepFiler® Express BTA™ extraction kit.

Currently, methods to extract DNA from bone, teeth, and adhesive substrates, such as cigarette butts, chewing gum, envelope flaps, and Fastape, are growing in popularity. However, there are multiple methods that give varying results when trying to extract DNA from these substrates. A possible reason for these variabilities could be the high rate of degradation/inhibition that occurs with bone, teeth, and adhesive substrates. PrepFiler® Express BTA™ DNA extraction kit was designed for those types of substrates because it is able to remove Polymerase Chain Reaction (PCR) inhibitors and helps extract more DNA from these substrates than previously used reagents when accompanied by an AutoMate Express™.

The validation for PrepFiler® Express BTA™ DNA extraction kit was conducted by the Oakland County Sheriff's Office Biology DNA Laboratory. During this validation, four different studies were performed to help determine if this extraction kit would be beneficial to implement for casework when working with these substrates. These included: (1) assess the optimal amount of sample from various types of bone/teeth, as well as determine the optimal incubation time for extraction; (2) confirm that all four of the AutoMate Express™ instruments in use in the lab give comparable results; (3) determine if PrepFiler® Express BTA™ is able to produce STR profiles from non-probative samples that are typical for case work and are suitable for comparison to known samples; and (4) determine if PrepFiler® Express BTA™ was better at removing inhibitors and extracting DNA from these challenging substrates when compared to PrepFiler® Express™. Along with these validation studies, a case study was performed on bone chips discovered in Italy that were believed to be from the 1800s. All samples were extracted on an AutoMate Express™ instrument using PrepFiler® Express BTA™, except for the samples that were extracted with PrepFiler® Express™. They were quantified using Quantifiler® Trio Quantification Kit on an Applied Biosystems® 7500 Real-Time PCR System and amplified using GlobalFiler™ PCR Amplification kit using an Applied Biosystems® 9700 GeneAmp® PCR System. Capillary electrophoresis was performed on Applied Biosystems® 3500 Genetic Analyzer.

It was determined during this validation that when using PrepFiler® Express BTA™, a complete STR profile was obtained for both femur and tooth powders from 10mg to 50mg, while rib powder was best extracted from 40mg to 50mg. However, using 50mg of powder obtained from the bone chips did not produce full profiles and produced only a partial STR profile for one of the ten samples. It was observed during this validation that when extracting DNA from multiple replicates of bone powder samples, all four of the AutoMate Express™ instruments did in fact produce similar profiles. Also, the validation illustrated that PrepFiler® Express BTA™ is able to produce profiles for non-probative evidence samples that can be used for comparison to known profiles. When comparing PrepFiler® Express BTA™ to PrepFiler® Express™, it was determined that PrepFiler® Express BTA™ gave overall better profiles with less degradation/inhibition when extracting DNA from adhesive substrate samples. However, when extracting DNA from Fastape, better quality profiles were obtained with extraction following the bone and tooth protocol instead of the adhesive substrate protocol.

Based on the results from the validation of PrepFiler® Express BTA™, it was concluded that this DNA extraction kit was able to extract DNA from bones, teeth, and adhesive substrates in a way that prevents PCR inhibition and increases the quantity of DNA extracted compared to PrepFiler® Express™. PrepFiler® Express BTA™ was able to extract DNA in a manner that produces STR profiles that can be used on forensic samples commonly processed in this laboratory.

**PrepFiler® Express BTA™, DNA Extraction, AutoMate Express™**





### E45 An Evaluation of the Effects of Various Digest Times on the DNA Yield for Differential Separations at the West Virginia State Police Forensic Laboratory

Allegra N. Forwith, BA\*, Marshall University Forensic Science Center, Huntington, WV 25703; Bailey E. Hill, MS, West Virginia State Police Forensic Laboratory, S Charleston, WV 25309; Kelly Beatty, MSFS, Marshall University Forensic Science Center, Huntington, WV 25701

**Learning Overview:** The goal of this presentation is to communicate the findings that 60 minutes is the ideal digest time for epithelial cell lysis in differential samples containing sperm from sexual assault cases. Attendees will learn that heating differential samples at 56°C for 60 minutes yields the best male DNA yield while maintaining the integrity of the DNA.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the method for processing sexual assault samples in order to obtain higher male DNA yields and better forensic male DNA profiles.

Every year, thousands of sexual assaults occur in the United States.<sup>1</sup> Sexual assaults make up a large percentage of the cases handled by forensic DNA laboratories and adopting an optimized procedure for processing these cases is of the utmost importance.

Differential extraction is the process used to separate epithelial cells (e-cell) and sperm cells during DNA extraction in sexual assault cases.<sup>2</sup> Samples are heated while suspended in digest reagents, with the goal of separating the sperm cells from the e-cells.<sup>3</sup> This isolated sperm can then be used to develop a male DNA profile.

The current procedure for differential extraction at the West Virginia State Police Forensic Laboratory requires sample digestion at 56°C for 30 minutes to two hours.<sup>2</sup> The purpose of this study was to determine the ideal incubation time within this range in order to optimize the DNA yield in the e-cell and sperm fractions of differential samples. Five digest times were evaluated in this study: 30 minutes, 45 minutes, 60 minutes, 90 minutes, and 120 minutes.

To prepare for this study, a three-fold serial semen dilution was prepared for a series of six semen dilutions (A-F). Female saliva was obtained and diluted (1:2) with nuclease-free water. Six sets of samples (A-F) were prepared by adding 25µL of a respective semen dilution and 25µL of saliva dilution to sterile cotton swabs and dried. Swabs were nutated in 1,000µL of nuclease-free water for one hour, transferred to spin baskets, and centrifuged at 13,000 RPMs for five minutes. The resulting liquid was added back to the sample tube and re-centrifuged for an additional five minutes to concentrate the cellular material into a pellet. Approximately 920µL of water was removed and discarded, and 500µL of digest mix (475µL Sarkosyl buffer + 25µL Proteinase K) was added to the remaining pellet for each sample.

Samples were digested on the 56°C heat block for each of the incubation times and transferred to the QIAGEN® QIAcube® for separation, using Protocol 12A/12B. A sperm-lysis solution was prepared for a pipet volume of 145µL per sample (7.25µL ProK + 7.25µL DTT + 130.5µL Sarkosyl buffer) and loaded into the QIAcube® in position A to be added to the sperm fraction after the wash steps. Samples were extracted on the QIAGEN® EZ1® using Large Volume protocol for the e-cell fraction and Trace protocol for the sperm fraction; samples were eluted in 40µL of TE buffer.

All samples were quantitated using Quantifiler® Trio on the Applied Biosystems® 7500 Real-Time PCR System and amplified using GlobalFiler™. Capillary electrophoresis was performed on the Applied Biosystems® 3500 Genetic Analyzer, and analysis was performed using GeneMapper® IDX.

Results for each semen dilution (A-F) indicate that a 60-minute digest time yields the highest male DNA quantitation values. The second highest male DNA yields were seen at 90 minutes or 45 minutes. The data for each semen dilution graphically follows a normal distribution pattern, with the ideal digest time being 60 minutes. Both the human and male DNA quantitation values for the 120-minute samples had the lowest quantitation values for all semen dilutions. The data suggests that 30 or 45 minutes is not enough time for the digest mix to lyse the e-cells.

The results generated from this study support the conclusion that 60 minutes is the ideal digest time. The DNA profiles from the 60-minute digest time samples had the purest and most robust sperm cell fractions, as they produced single-source profiles with alleles above the analytical threshold. The sperm fraction of samples digested at 30 and 45 minutes contained female DNA, likely due to undigested epithelial cells. The sperm fraction samples subjected to 90- and 120-minute digest times had good separation but showed evidence of degraded DNA due to lower allele peak heights.

While male DNA profiles can be generated from samples heated for 45–90 minutes, the ideal digest time for differential samples is 60 minutes. Implementing a strict 60-minute digest time into the differential separation protocol could lead to better separation of epithelial and sperm cells, while maintaining the integrity of the male DNA.

#### Reference(s):

1. Sexual Assault Forensic Examination (SAFE) Commission Annual Report. *West Virginia Division of Justice and Human Services*, (September 1, 2016-August 31, 2017): [http://www.wvlegislature.gov/legisdocs/reports/agency/J03\\_G\\_2017\\_13749.pdf](http://www.wvlegislature.gov/legisdocs/reports/agency/J03_G_2017_13749.pdf).
2. WVSPFL Quality Assurance Board. *The West Virginia State Police Forensic Laboratory DNA Analysis Procedures Manual*. (July 2018), South Charleston, WV.
3. Marshall University Forensic Science Center DNA Laboratory. *Internal Validation of the QIAcube for Differential Separation*. (May 2013), Huntington, WV.

#### Differential Extraction, Sexual Assault, Digest Time



### E46 The Effect of Crime Scene Investigation (CSI) on Forensic DNA Database Recognition: A Public Survey of South Koreans

Jihyun Lee, Seoul, SOUTH KOREA; Sung Kyum Cho, PhD, Chungnam National University, Daejeon 34134, SOUTH KOREA; Sohee Cho, PhD, Seoul National University College of Medicine, Seoul 110-799, SOUTH KOREA; Moon-Young Kim, Seoul National University College of Medicine, Seoul 03080, SOUTH KOREA; Soong Deok Lee, PhD, Seoul 110-799, SOUTH KOREA; Kun Won Jung, MD, PhD\*, Hanyang University, Seoul, SOUTH KOREA

**Learning Overview:** After attending this presentation, attendees will understand the impact of forensic television programs such as *CSI* on overall forensic awareness, including forensic investigation and the DNA database, through a survey of South Koreans.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how people are affected by media, including forensic science television programs, and how people's perceptions change. A survey of South Koreans' attitudes affected by media demonstrates a change in opinion regarding forensic science overall, especially the criminal DNA database.

Data was collected as a part of the Korean Academic Multimode Open Survey for Social Sciences (KAMOS), which provides scientific survey data for social scientists. KAMOS is a member of the international Open Probability-Based Panel Alliance (OPPA). KAMOS is a representative survey of Korean adults. The panel was created using stratified cluster random sampling of home addresses. Of 2,000 randomly selected panel members, 1,000 respondents participated in this survey.<sup>1,2</sup>

The survey on which we asked our questions also saw a rise in the popularity of crime procedural dramas in Korea, such as *CSI* and *Law and Order*. These dramas portray forensic teams and detectives in a positive light and demonstrate how having a database of information available in criminal situations and persons may help solve crimes. Of the respondents, 57.8% (more than 50% of all ages, except 60 or older) responded that they have seen forensic-related media contents. In addition, a majority of these respondents had higher education than a high school diploma. It is believed that there are barriers, such as linguistic or scientific education, in encountering foreign media or in understanding forensic science and scientific facts. The 578 people who had seen forensic dramas or movies were asked how their perception of forensic investigation methods changed after watching the video; 70.5% of respondents thought they were more positive than before. Only 4.7% of respondents thought they were more negative than before.

Between similar surveys conducted in 2003 and this survey, there have been many series about crime scene investigation in Korea. This study may provide a comparative analysis of the results of two surveys over time and the opinions of those exposed to these media have changed. We may also be seeing some version of the "CSI Effect" in Korea as well; that is, there may have been an increase in positive attitudes toward the use of a database thanks partially to these programs. While a survey from the United States found that watching crime shows such as *CSI* was positively correlated with a belief in the reliability of DNA testing, it was not related to support of a DNA bank. Nonetheless, the "CSI Effect" may be slightly different in Korea and may provide a partial explanation for the increase in positive attitudes toward a criminal DNA database.

This survey will help enlighten policymakers about what to consider when linking forensic science and legal systems. Public opinion is also an important consideration when enacting or amending the law, and it is necessary to prevent public opinion from being distorted by media exposure.

#### Reference(s):

1. Baskin D.R., Sommers I.B. Crime-Show-Viewing Habits and Public Attitudes Toward Forensic Evidence: The "CSI Effect" Revisited. *Justice System Journal* 2010;31(1):97-113.
2. Weaver R., Salmonson Y., Koch J., and Porter G. The CSI Effect at University: Forensic Science Students Television Viewing and Perceptions of Ethical Issues. *Australian Journal of Forensic Sciences*. No. 44, 4 (2012): 381-391.

**"CSI Effect", Public Survey, Forensic Science**



### E47 An Evaluation of the Efficacy of Whole Genome Amplification (WGA) for Degraded DNA

Mitsuyo Machida\*, Tokyo Women's Medical University, Tokyo 162-8666, JAPAN; Kazuhiko Kibayashi, MD, Tokyo Women's Medical University, Tokyo 162-8666, JAPAN

**Learning Overview:** After attending this presentation, attendees will understand the need for Whole Genome Amplification (WGA) prior to Short Tandem Repeat (STR) analysis using degraded DNA.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that WGA is a potential preamplification method for STR analysis of challenging DNA samples.

DNA in biological samples is often vulnerable to environmental factors, such as Ultraviolet (UV) irradiation, high temperature, high humidity levels, and microorganism degradation. STR profiling, the most commonly used method for forensic DNA identification, is difficult to analyze highly degraded DNA samples, since structural modification or fragmented DNA templates can affect downstream analyses. An increase in the number of starting templates may improve the success of STR profiling. One approach to increase the number of DNA templates is WGA. Although WGA has mainly been demonstrated in clinical applications, few studies have shown the ability to evaluate the availability of WGA for degraded DNA samples in forensics.

Therefore, a PCR-based WGA called modified Improved Primer Extension Preamplification (mIPEP) was performed prior to STR analysis using degraded DNA, since the PCR-based WGA method is less affected by DNA quantity and quality. The mIPEP method uses a 15-mer random-sequenced primer and low stringency annealing conditions to amplify larger templates of the genome. To assess the efficacy of the mIPEP method for degraded DNA, this study artificially degraded DNA using UV light for different durations and analyzed the STR profiling.

Saliva from four volunteers was previously dried onto filter papers. These samples were exposed to UV light (365nm) for 3, 7, and 14 days, and 1 month in duplicate. The DNA were extracted from UV-irradiated saliva samples. The mIPEP method was initiated using 5ng and 0.5ng of DNA. Following the mIPEP, STR analysis was performed using the AmpF $\ell$ STR<sup>®</sup> Identifiler PCR Amplification Kit. This study was approved by the ethics committee at Tokyo Women's Medical University.

The average peak height of 16 STRs drastically decreased until seven days of irradiation, then gradually decreased until one month of irradiation without mIPEP. After performing mIPEP using 5ng of input DNA, the peak height was higher than that without and with mIPEP using 0.5ng of input DNA after seven days of irradiation. Successful STR profiling decreased with increasing duration, and 12% of the STR loci were shown after one month of irradiation without mIPEP. The number of detectable STRs with mIPEP using 0.5ng of DNA was similar to that without mIPEP after UVA irradiation. However, the number of detectable STRs with mIPEP using 5ng of DNA was greater than that without and with mIPEP using 0.5ng of input DNA after 14 days of irradiation. In addition, longer STR loci, such as D21S11, D7S820, CSF1PO, D2S1338, D18S51, and FGA, were difficult to recover despite performing mIPEP.

Hence, 5ng of input DNA is necessary for mIPEP to improve the success of STR profiling using highly degraded samples. Moreover, the difficult recovery of STR loci by mIPEP should be considered when analyzing STRs using degraded DNA.

#### Whole Genome Amplification, Short Tandem Repeat, Degraded DNA



**E48 An Optimized Small Molecule Extraction Method for Dried Blood Spots (DBS)**

*You-Rim Lee, BS\*, Gyeonggi-do, Korea, SOUTH KOREA; Jiyeong Lee, PhD, Eulji University, Seongnam-si 13135, SOUTH KOREA; Yoo-Jin Lee, MT, Eulji University, Gyeonggi-do 13135, SOUTH KOREA; Hyo-Jin Kim, MT, Eulji University, Gyeonggi-do 13135, SOUTH KOREA; Sora Mun, MT, Eulji University, Gyeonggi-do 13135, SOUTH KOREA; Hee-Gyoo Kang, PhD, Eulji University, Gyeonggi-do 13135, SOUTH KOREA*

**THIS ABSTRACT WAS NOT PRESENTED.**



### E49 The Development of Two Forensically Important Blow Flies Collected From Human Remains in Western Colorado

Brandon V. Everhart, BS\*, Cornell University, Department of Entomology, Ithaca, NY 14853; Alexander J. Smith, BA, Grand Junction, CO 81502; Melissa A. Connor, PhD, Colorado Mesa University, Grand Junction, CO 81501-3122; Elson Shields, PhD, Cornell University, Entomology Department, Ithaca, NY 14853

**Learning Overview:** The goal of this presentation is to provide attendees with information regarding the necrophagous flies that colonize human remains in western Colorado, with an emphasis on the development of two species poorly represented in the literature.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the two dominant species of blow flies found during the late fall to early spring months at the Forensic Investigation Research Station (FIRS) located in Mesa County, CO. Data on the two fly species, *Calliphora latifrons* and *Calliphora coloradensis*, are either poorly represented or absent in the literature, and this research will help fill an important information gap. This developmental data will also be useful throughout the multi-state range for these two species.

Necrophagous insects provide a wealth of information during death investigations. This information includes time since death, whether neglect or abuse took place antemortem, and if remains were moved.<sup>1</sup> In particular, flies in the family Calliphoridae are generally the first insects to visit remains and are usually the most forensically informative. They are often used to estimate the Time Of Colonization (TOC) and, thus, the Postmortem Interval (PMI). However, to properly use insects for this purpose, it is important to know which necrophagous insects are present in a given area and the rate at which they develop.

FIRS is a facility dedicated to researching human decomposition. FIRS represents the only such facility in the semi-arid region of the southwestern United States, providing a unique opportunity to study human decomposition under these conditions. The presence of donated human remains placed in the facility throughout the year allows for a continuous survey of blow fly species active during the different seasons of the year.

Cadavers were inspected daily at FIRS and maggots were collected when seen on cadavers. To identify the blow flies that colonized human remains in the area, maggots were collected and reared out using methods identified in the FIRS protocols.<sup>2</sup> Pupae and deceased adult flies were sent to Cornell University, where they were identified to species. Two species, *Calliphora latifrons* and *Calliphora coloradensis*, were found to be the dominant colonizers of human remains during the late fall to early spring (November–March). The lack, or minimal amount, of developmental data in the literature for these two species suggested that a basic study on temperature-related development would be useful to the forensic community, so this project developed a study.

Development trials were completed at Cornell University's Shields Laboratory using reach-in environmental chambers set at different temperatures as replicates. Initially, development was documented across a broad range of temperatures (15°C, 20°C, 25°C, and 30°C). This was followed by more focused trials looking at development at a low range of temperatures (4°C, 8°C, 11°C, and 13°C). These lower temperatures were chosen to reflect the common ambient temperatures at the FIRS facility during the seasons when *C. latifrons* and *C. coloradensis* are most common. Three replicates were run at each temperature with each replicate consisting of four enclosures where maggots were allowed to develop from egg to adult with sampling conducted at regular intervals to measure maggot length and determine life stage. The resultant data was used to make isomegalen and isomorphen diagrams as well as a thermal summation model. Validation trials are planned to test the lab-collected temperature data in a field environment at the FIRS facility.

Findings generated from this work provide important baseline information regarding insect activity at the FIRS facility. This information will allow FIRS to more fully assist law enforcement in the area and pave the way for future experiments exploring the human necrobiome and the development of other insects that colonize human remains at the FIRS facility. It has ramifications for estimation of the PMI throughout the semi-arid areas of the western United States.

#### Reference(s):

1. Greenberg, B. Flies as Forensic Indicators. *Journal of Medical Entomology*. 1991 (28) 565-577.
2. Smith, A.J. Entomology Protocols. *FIRS Technical Manual 5*. Series editor, M. Connor. Forensic Investigation Research Station, Colorado Mesa University, Grand Junction CO. 2018.

#### Forensic Entomology, Blow Flies, Development



### E50 The Effects of Ketamine and Xylazine on Larval Development in the Blow Fly (*Phormia Regina*)

Stephanie A. Olson\*, Arcadia University, Glenside, PA 10938; Tobias Landberg, PhD, Arcadia University, Glenside, PA 19038; Brandon Strauss, MS, University of Nebraska-Lincoln, Lincoln, NE 68588; Karen S. Scott, PhD, Arcadia University, Glenside, PA 19038

**Learning Overview:** After attending this presentation, attendees will better understand how the presence of ketamine and xylazine in the diet of black blow fly (*Phormia regina*) maggots affects their growth and development throughout the larval stages.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing entomological data to increase the accuracy of Postmortem Interval (PMI) determinations.

Entomotoxicology is an expanding field of forensic entomology that focuses on the effects of drugs and other toxins on insects. This project spans both main branches of entomotoxicology, growth/development studies and extraction/qualitative studies. This study focuses on development and will report the extraction results elsewhere. Insect larvae also produce a unique opportunity for toxicological samples when all other body tissues and fluids have degraded in late stages of decomposition. Previous studies have shown that there are various effects of drugs on the growth and development of insects. For example, ketamine and xylazine are sedatives that are commonly used in veterinary medicine. Ketamine is a common party drug that is known on the street as Special K. It is also associated with drug-facilitated crimes. Xylazine is not commonly abused on its own, but it has been found in street heroin samples. Since these two drugs are both found on the street, they are of forensic importance. Asian blow fly, (*Chrysomya megacephala*) development may be affected when reared on rat meat dosed with ketamine and xylazine compared to those reared on drug-free meat.<sup>1</sup> These differences can lead to errors in PMI estimation. However, development may speed up or slow down depending on drug dose and the stage of larval development.

Preliminary research conducted on flesh-eating beetles (*Dermestes maculatus*) indicated there was no development change when the beetle larvae were exposed to ketamine in their rat meat diet. The purpose of this experiment is to evaluate temporal dynamics of larval instar-related developmental changes when exposed to ketamine and/or xylazine in a North American blow fly, *P. regina* (as overall developmental rate may not be affected).

*P. regina* is a cosmopolitan species that is commonly encountered and utilized in forensic entomology. The present study describes the effects of ketamine and xylazine on the development of *P. regina* from first to third instar. Twenty Sprague Dawley rats were dosed with ketamine and/or xylazine in saline via intraperitoneal injection as follows: K1 at 100mg/kg of ketamine, X1 at 10mg/kg of xylazine, K2X2 at 300mg/kg ketamine and 30mg/kg of xylazine, and K3X2 at 450mg/kg of ketamine and 30mg/kg of xylazine. The first two doses are therapeutic levels and the last two are lethal doses. A control group was injected with saline. All rats were then euthanized and dissected; the brain, liver, and heart were removed. Ten maggots were then placed onto each organ, and larval stage was recorded twice per day. Temperature was monitored to determine Accumulated Degree Hours (ADH) to provide a more accurate measure of PMI.

The results indicate that there is an effect on early instar developmental rate (instars 1 and 2) which is drug- and dose-dependent; however, larval development (to instar 3) is similar to controls. This highlights the importance of determining drug presence in tissues and understanding instar-dependent variation of PMI estimations utilizing *P. regina*.

#### Reference(s):

1. Singh, D., Heer, B.K., and Wadhawan, B. Effect of Ketamine Hydrochloride in Combination with Xylazine on the Development of *Chrysomya megacephala* Fab. *Indian Journal of Forensic Medicine & Toxicology*, vol 8(1), (January 2014):140-144.

#### Entomotoxicology, Ketamine, Xylazine



## E51 Profiling the Odorant Chemicals Present in *Cimex Lectularius*

Katelynn A. Perrault, PhD\*, Chaminade University of Honolulu, Honolulu, HI 96816

**Learning Overview:** After attending this presentation, attendees will better understand the Volatile Organic Compounds (VOCs) emitted from *Cimex lectularius* (bed bugs), a well-known human parasite that is commonly detected by scent detection canines in the United States.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by studying the scientific foundation for the use of pest/insect detection canines, which is important due to the current development of guidelines surrounding their use by the Organization of Scientific Area Committees (OSAC).

In 2017, the Dogs and Sensors Subcommittee within the Crime Scene/Death Investigation Committee of OSAC began to establish guidelines on the training and certification of pest/insect detection canines. The goal of these guidelines is to improve the performance, reliability, and courtroom defensibility of detection dog teams in the field. Current best practices dictate that training shall be performed on actual live *C. lectularius* adults. There is currently a major knowledge gap in the VOCs produced by *C. lectularius*, and limited current research being conducted in this topic area. Surrogate training aids are available for *C. lectularius*; however, these are not recommended by current practices. In addition, very little is known about the difference in odor profile between live and dead *C. lectularius* adults, as well as the various life stages of this species. The overall aim of this work is to provide chemical data that can improve detector canine operational performance and provide a scientific foundation for training, certification, and use guidelines. The objective of the current study was to establish a VOC profiling method for *C. lectularius* that can be used in future work and to provide a preliminary comparison of living and dead *C. lectularius* adults.

Adult *C. lectularius* were profiled in sealed headspace vials using Solid-Phase Microextraction (SPME) Arrow. This is a technique that permits non-contact sampling of the insects, while allowing a high capacity of VOC loading onto the Arrow fiber. The samples were then analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). A VOC signature could be detected from two adults in a single vial; however, a 10min exposure time was recommended to increase the intensity of the signal for GC/MS analysis. This preliminary work identified that some compounds were specific to live *C. lectularius* (e.g., phenol, 6-methyl-5-hepten-2-one) and some were specific to dead *C. lectularius* (e.g., 2-octenal). In addition, some compounds were found to be in common between the live and dead insects but were found either in higher concentrations in the live individuals (e.g., decanal, caprolactam) or dead individuals (e.g., 2,4-ditertbutylphenol). A single compound, nonanal, was found to be in both live and dead bed bugs at approximately equal concentrations. Additional work is needed to validate the identity of these specific compounds and whether these trends can be consistently seen across insects that may have been bought from different sources or have been stored for different lengths of time. Improving this chemical foundation for the use of live *C. lectularius* insects during detection dog training may assist in developing improved or more convenient training procedures. It may also permit the development of suitable surrogate training aids.

This research is significant because it is the first primary study that provides a chemical foundation for the use of live *C. lectularius* for training bed bug detection canines. This can assist in improving standards for training, certification, use, documentation, and management of detection canines as a valuable forensic tool. It will also serve to improve the reliability and courtroom defensibility of pest/insect detection canines.

### Scent Detection Canines, Volatile Organic Compounds (VOCs), Bed Bugs

## E52 Can Body Cooling Animal Models Help Solve the Jeju Island Cold Case?

*Dae-Kyoon Park, MD, PhD\**, Soonchunhyang University, Cheonan-si, Seoul 31151, SOUTH KOREA; *Duk-Soo Kim, PhD*, Soonchunhyang University, Cheonan-si, YT Seoul 331946 Korea, SOUTH KOREA; *Cheolho Hyun*, Jeonju, Jeonbuk, SOUTH KOREA; *Taehwa Song*, Korean Police Investigation Academy, Asan, SOUTH KOREA; *Kyeongyang Sim*, Suweon, Gyeonggido, SOUTH KOREA; *Na Jin Kim*, Busan, SOUTH KOREA

**Learning Overview:** After attending this presentation, attendees will understand the application of body cooling animal models to solving cold cases in that the rectal temperature could be influenced by the ambient temperature and the surface temperature where the body was laid.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating more reliable time-since-death estimation in the early stages of decomposition by employing animal models.

When a body is found at the early stages of decomposition, crime scene investigators estimate time since death using the Henssge nomogram that requires rectal and ambient temperature. However, the application of the Henssge nomogram is problematic in Korea because there may be interferences with body heat radiation during algor mortis. A previous study suggested that the temperature of the substrate has a greater effect on the postmortem cooling of the body than the ambient temperature.<sup>1</sup> For this experiment, key words in cold case files such as “rectal temperature” or “time since death” were reviewed. The Jeju Island homicide case involves a body that was found in a drainage area at 1:30 p.m., on February 8, 2009, seven days after the victim was reported missing. Six hours later, the body was moved to the road beside the scene, and the rectal and ambient temperatures were 13°C, and 9.2°C, respectively. According to the autopsy report, internal organs were fresh, and stomach contents were present. Based on these observations, the postmortem interval was estimated as within 24 hours from the time the body was found. The prime suspect was released from the police investigation as the man had an alibi during that postmortem interval. The purpose of this study is to reconstruct the Jeju Island crime scene and to reevaluate the postmortem interval by employing animal models.

The study was approved by the Korean Police Investigation Academy Institutional Animal Care and Use Committee (KPIA-18—01). Four pig (*Sus scrofa* L.) and three beagle (*Canis familiaris* L.) carcasses were placed at the scene where the body was found and monitored for eight days by Closed-Circuit Television (CCTV), from January 29 to March 2. Six temperature probes were placed: inside the rectum; on the body surface (skin); inside the clothing; on the surface of the clothing; in between the body surface and the surface of the drainage; and inside the drainage. Temperatures were recorded every ten minutes with a data logger system, and the WatchDog® 2000 mini station was placed next to the carcasses to record ambient temperature.

The mean temperature inside the drainage area was higher (0.93°C to 3.13°C) than the mean ambient temperature during the daytime. Because the drainage was exposed to sunlight from 9:00 a.m. to 4:00 p.m., the temperature inside the drainage might be affected by solar radiation. The rectal temperature reached that of the ambient temperature in at least 50 hours in pigs and 28 hours in beagles. The rectal temperature fluctuated thereafter; as the temperature inside the drainage rose, the rectal temperature rose four to six hours after, and when the temperature inside the drainage fell, the rectal temperature fell six to eight hours after. Therefore, the Henssge nomogram may have been an unreliable method for estimating postmortem interval in the Jeju Island homicide case.

The present study demonstrates that the postmortem interval in the Jeju Island case is greater than 24 hours because the victim’s rectal temperature was possibly affected by the temperature inside the drainage. Furthermore, the victim was found seven days after being reported missing. After reviewing the original autopsy report and applying the results of this study, it was estimated that the postmortem interval is 28 to 50 hours from the day the victim was reported missing and not from time when the body was found. It could also be suggested that the Jeju Island case needs to be reinvestigated. The results of this study on postmortem cooling of animal models has a potential application in unsolved cold cases in Korea.

### Reference(s):

1. Park D.K. et. al. Estimation of Time Since Death Using Body Cooling Models of Pigs: A Pilot Study. *Proceedings of the American Academy of Forensic Sciences*, 69<sup>th</sup> Annual Scientific Meeting, New Orleans, LA. 2017. E41.

### Postmortem Interval, Henssge Nomogram, Rectal Temperature





### E53 Climate's Influence on Diptera Present on a Carcass

*Timothy Juedes, MS\**, Platteville, WI 53818; *Ismail M. Sebetan, MD, PhD\**, National University, La Jolla, CA 92037-1011; *Paul Stein, PhD\**, National University, La Jolla, CA 92037

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**Learning Overview:** After attending this presentation, attendees will better understand the role of climate on the presence of flies (Order: Diptera) that would be expected to colonize human remains in southwest Wisconsin.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing the relationship between climate and the presence of flies on remains located in southwest Wisconsin. Entomological information is provided from other studies conducted that have similar climates to southwest Wisconsin for data analysis. Relationships between data collected from the adult and larval flies recovered from pig and rabbit carcasses and the Postmortem Interval (PMI) will be presented.

This study hypothesized that the local climate would affect the Diptera present on a decomposing carcass. A key objective was to identify the taxonomic relationships of flies (Order: Diptera) that colonized the pig and rabbit carcasses. The locations of these flies were documented relative to the proximity to areas of trauma. Postmortem indicators of death (algor, livor, and rigor mortis) were also observed and recorded. A scale was developed to determine the level of fixation for each of the PMI indicators observed.

Animal carcasses were exposed to the local climate conditions recorded between September 2016 and December 2017 (16 months) at the field study station located in Platteville, WI. The animal was placed on a soil-filled wooden box that was placed on top of a cement slab isolated by a chain link kennel fence. This simulated a body dump site on top of soil but was protected from scavenging animals. Temperatures were measured at selected intervals up to 72 hours after death. Fly samples were collected at these intervals and at any time the site was inspected. A chi-square test for independence was used to analyze the various data collected and significant differences indicated by p values <0.05

Results of the study indicated that the climate impacted the presence and species of flies colonizing the animal carcasses at the field station. Two families of Diptera were present, these were identified as Calliphoridae and Muscidae. Data also agreed with the observations that flies appear on deceased bodies prior to any other indicators of death. The data indicated that flies did not have a preference to colonize an area of trauma or a natural orifice. Temperature and rainfall impacted the quantity of flies and number of species present in the warmer months (June–September) compared to the colder months (October–December) when flies were noticeably reduced.

Entomological information on the presence and species of flies colonizing remains found in southwest Wisconsin was reported for the first time by these research findings. This information may be extrapolated to human remains and would be a basis for interpreting medicolegal findings during possible homicide investigations.

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**Calliphoridae-Muscidae, Climate, Forensic Entomology**



### E54 The Mystery Behind the Composition of Black Fingerprint Powders

*Grayce Behnke, BS\*, Export, PA 15632; Andrea Kardohely\*, Canton, OH 44718; Catherine G. Rushton, EdD, Marshall University Forensic Science Program, Huntington, WV 25701; Rosalynn Quinones, PhD, Marshall University, Huntington, WV 25755*

**Learning Overview:** After attending this presentation, attendees will understand the characterization of black fingerprint powders using a variety of different analytical instrumental techniques in order to determine the particle size, homogeneity, elemental composition, and surface functionalized groups.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by classifying black fingerprint powders in order to assist in the selection of a fingerprint powder that is most appropriate for the desired use. The objective of this research was to characterize black fingerprint powders in order to assist latent print examiners in the selection process of a black powder for fingerprint development.

Fingerprint development has been used to visualize latent prints since the 19<sup>th</sup> century, and several modern companies produce a variety of different commercially available black fingerprint powders. While the method to develop fingerprints has been refined over the years, the composition of the fingerprint powders that are used in this print development has not been studied extensively.

Black fingerprint powders were analyzed using Dynamic Light Scattering (DLS), zeta potential, Inductively Coupled Plasma/Optical Emission Spectroscopy (ICP/OES), Attenuated Total Reflectance/Infrared (ATR/IR) spectroscopy, and Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray Spectroscopy (EDS). DLS can be used to determine the size distribution of small particles in suspension or solution. Zeta potential can be used to evaluate the stability of particles in solution. ICP/OES is an analytical technique that is used in the detection of chemical elements. ATR/IR can be used to identify the functional groups present in a sample with little to no sample preparation. SEM is used to produce images of a sample by scanning the surface with a focused beam of electrons. EDS is used to determine the elemental composition of a sample.

DLS was used to obtain the particle sizes of the black fingerprint powders in an aqueous suspension and SEM was used to obtain the particle sizes of the black fingerprint powders in their native form. DLS and SEM in conjunction were used to assess the homogeneity of the fingerprint powders. ATR/IR assisted in determining the surface functional groups present in the variety of black fingerprint powders studied. ICP/OES was used to quantify the elements present and, in conjunction with EDS, was used to determine the elemental composition of the components present in black fingerprint powders.

Preliminary DLS data indicates that black fingerprint powders from several different manufacturers are not homogenous and that the dispersion of particle sizes in the samples varies between different manufacturers. It should be noted that the average particle size remains fairly consistent between manufacturers. While the particle size dispersion varies between manufacturers, the zeta potential, which is the surface charge of the particles, is consistently negative and ranges from -26.31mV to -37.31mV. This indicates that the surfaces of the particles are stable in an aqueous solution. The methods of characterization described above will be applied to colored fingerprint powders, in addition to white and bichromatic fingerprint powders, in the future.

Black fingerprint powders from different manufacturers can be characterized using DLS, zeta potential, ICP/OES, ATR/IR, and SEM with EDS. This characterization can be used to assess the performance of black fingerprint powders in the development of latent prints.

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#### Fingerprint Powder, Characterization, Particle Size



### E55 A Comprehensive Schematic for Postmortem Fingerprint Stations at Mass Fatality Incidents

*Marzena H. Mulawka, MFS\*, San Diego County Medical Examiner's Office, San Diego, CA 92123; Michael A. Mosco, MS\*, San Diego Police Department Crime Lab, San Diego, CA 92101; David Martinez, BS, San Diego, CA 92117*

**Learning Overview:** After attending this presentation, attendees will better understand how to set up a comprehensive disaster morgue fingerprint station for Postmortem (PM) fingerprint recovery during Mass Fatality Incidents (MFIs). A schematic of the fingerprint station will be displayed, along with various types of supplies and equipment that may be used for obtaining examination-quality fingerprint records from Unidentified Human Remains (UHR). Incorporating the highlighted equipment and supplies into disaster morgue operations may help identify many of the UHR that are processed during MFIs. Not only will identification of UHR yield valuable investigative information, but it will also allow for the notification of next-of-kin regarding the fate of their family member.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping further the ability of forensic and emergency management professionals to enhance disaster morgue operations by utilizing the comprehensive PM fingerprint station to efficiently and effectively handle fingerprint identification matters during disasters, especially for agencies exhibiting a high caseload during MFIs, when time and resources are significantly limited. This presentation will provide the specific resources needed to set up the presented PM fingerprint station during MFIs.

The use of fingerprints for forensic identification is a rapid, reliable, and cost-effective means to identify UHR. The use of friction ridge impressions for forensic identification during MFIs is well established in forensic science. However, the most efficient workflow for PM fingerprint recovery in a disaster morgue setting can be complex, often requiring the need for a more comprehensive and complete PM fingerprint station containing various specialized equipment and supplies. Factors such as the scale and nature of the incident, condition of the various remains, and condition of the friction ridge skin on each decedent are rarely uniform and often unexpected. These factors will dictate which methods must be used to successfully enhance and record any valuable friction ridge information. As a result, multiple techniques exhibiting varying complexities may be used with each set of remains. The wide range of techniques include, but are not limited to, the reconditioning of skin using tissue injection, soaking/rehydration, boiling, manipulation of degloved epidermal skin, as well as recording techniques, such as ink/card, fingerprint powder/adhesive lifter/acetate sheet, fingerprint powder/castings, photography, and digital scanning. As such, the appropriate supplies and equipment for each of the various techniques should be readily available in the disaster morgue. This can be accomplished by following the schematic and creating a comprehensive inventory of PM fingerprint recovery supplies and equipment that can be rapidly deployed during MFI disaster morgue operations.

#### Postmortem Fingerprint Recovery, Mass Fatality Incidents, Forensic Identification



### E56 Forensic Podiatric Science and Practice in Crime Scene Investigation

*Kewal Krishan, PhD\*, Panjab University, Chandigarh 160 014, INDIA; John A. DiMaggio, DPM, Bandon, OR 97411-8816*

**Learning Overview:** After attending this presentation, attendees will understand the value of an important and emerging subdiscipline of forensic science, forensic podiatry, which deals with the examination, interpretation, and evaluation of pedal evidence encountered at crime scenes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the utility of a comparatively new subsection of forensic science that is concerned with the inspection of pedal evidence at crime scenes. This may motivate young forensic scientists to take up this discipline for research and practice.

Forensic podiatry is comparatively a new scientific subdiscipline of forensic science that deals with the examination of pedal evidence generally encountered at crime scenes. It is defined as the application of sound and researched podiatric knowledge and experience in forensic investigations to show the association of an individual with a scene of crime or to answer any other legal question concerned with the foot or footwear that requires knowledge of the functioning foot.<sup>1</sup> Forensic podiatrists contribute to the personal identification in crime scene investigations whenever foot-related evidence is recovered from the crime scene. The need to establish the identity of dismembered remains may arise in cases of mass fatality incidents such as terrorist attacks, mass murders, transport accidents, tsunamis, floods, and earthquakes. Dismembered and mutilated remains are usually encountered in these mass fatality incidents. There is an increased likelihood of the recovery of feet (often enclosed in shoes), separated from the body in mass disasters such as high-power explosions and bomb blasts, airplane crashes, and other high-impact transportation accidents. In this regard, forensic podiatrists can collect the evidence related to feet and help in the identification of the individuals from the foot and its parts. Furthermore, forensic podiatrists conduct the examination of footprints generally recovered at the crime scene. Footprints are commonly recovered at every crime scene in the form of bare footprints, socked footprints, or shoe prints.

There are many ways in which footprints can be used to establish personal identification in forensic podiatry. The analysis of bare footprints involves identification based upon class, intermediate class, and individual characteristics of the footprints. For example, features such as corns, pits, ridges, humps, creases, hammertoe deformity, an extra toe, missing toe in the foot impression, and flat footedness are characteristics of the footprints that can be utilized as forensic evidence in establishing personal identification. This type of physical evidence can positively link a suspect to a crime, or it can prove one's innocence. By using different anthropometric methods, the stature, sex, and body weight can also be estimated from the footprints recovered at the crime scene. As stature and body weight can provide an idea about the size of the individual, so can they provide useful clues to a forensic scientist in a criminal investigation. Apart from this evidence in forensic podiatry, gait analysis and step/stride length analysis can also furnish some indication about the criminals involved in a particular case.

This presentation will discuss various methods of personal identification related to the pedal evidence which is usually recovered from the crime scene or scene of occurrence in the form of mutilated/dismembered remains, footprints, or questioned footwear.

**Reference(s):**

<sup>1</sup>. DiMaggio J.A., Vernon W. *Forensic Podiatry—Principles and Methods*. CRC Press, Taylor and Francis Group, Boca Raton, Florida 2017.

**Forensic Podiatry, Crime Scene Investigation, Personal Identification**



### **E57 A Reference Collection of Footwear Randomly Acquired Characteristics (RACs) for Frequency and Spatial Distribution Analysis**

*Kim Dang, BS, ORISE/FBI, Manassas Park, VA 20111; Colbey Ryman\*, Woodbridge, VA 22191; Thomas G. Kopczynski, BS\*, Mokena, IL 60448; Katherine Ky, ORISE/FBI, Oak Ridge, TN*

**Learning Overview:** After attending this presentation, attendees will understand how RACs can be used to determine frequency and spatial distributions for probability assessments in forensic footwear analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a dataset to be used for statistical analyses and determining RAC probability assessments.

Footwear impressions are often deposited on a surface when a shoe worn by an individual makes contact with a surface. If detected and recovered, this evidentiary item can provide valuable investigative linkage to a suspect. RACs are frequently used to make comparisons between a questioned impression and known footwear. Recently, there have been courtroom challenges regarding the discrimination potential of footwear evidence, particularly when there are mass productions of similarly modeled and sized shoes. To address these challenges, the Federal Bureau of Investigation (FBI) is currently compiling data on the frequency and spatial distribution of RACs detected on the outsoles, then marked using Adobe® Photoshop® for more than 1,500 boots with a similar outsole pattern and class characteristics. A visualization of common and rare RAC locations and frequencies are represented in heat maps, providing a visual approach into the role RACs play in the differentiation of impressions and outsoles.

The purpose of this study was to assign and characterize, in a quantitative fashion, the frequency and positional descriptions of RACs on boot outsoles acquired by FBI agents in training as they performed similar tasks while wearing the same type of Eastern Mountain Sports® Day Hiker boots.

To prepare boots for analysis, the outsoles were initially cleaned and scanned. Test impressions of the outsoles were created with the use of black powder and adhesive sheets. The scanned boot impression and corresponding test impression were then aligned with each other. After background subtraction, a comparable boot shape was obtained.

Preliminary visual interrogations using only oblique lighting of each physical boot outsole were conducted after test impressions were created. Documentation of any discernible RACs were recorded on a paper template without magnification. Observations of the outsoles of the boots were made in conjunction with the corresponding scanned test impressions to assess that all of the lugs were impressed properly. When the impression was verified, Quality Assurance/Quality Control (QA/QC) checks for both intra- and inter-evaluator RAC assessments were completed.

Throughout the project, questions were raised about RAC frequency and placement. While there was no correlation between RAC counts and boot size, greater percentages of RACs were found in the toe and on the edges than in other areas of the boot. Results also indicate there is no correlation between RAC frequency and gender. Since weight, height, and gait were all unknown variables, they were not considered.

The FBI boot outsole reference collection dataset consists of more than 150,000 RACs and provides valuable information for quantifying the discrimination potential of footwear evidence, specifically in regard to the use of RAC location in outsole identifications. Shape similarity metrics will be applied in Phase II of this project to quantitatively compare the closeness of features across known non-match boots to evaluate the probability of two different boots sharing the same shaped feature or feature set.

#### **Footwear Analysis, Randomly Acquired Characteristics, Database**



### E58 A Longitudinal Database of Athletic Shoe Outsole Wear

*Susan VanderPlas\*, Ames, IA 50011; Guillermo Basulto-Elias, PhD, Iowa State University, Ames, IA 50011; James E. Kruse, CSAFE, Iowa State University, Ames, IA 50010; Stacy Renfro, MS, Iowa State University, Ames, IA 50011; Alicia L. Carriquiry, PhD, Center for Statistics and Applications in Forensic, Ames, IA 50011*

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**Learning Overview:** After attending this presentation, attendees will be familiar with the features of a new longitudinal database of athletic shoe outsoles. Attendees will be able to query the database for data from different collection methods, shoe styles and sizes, and wear levels.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on a new resource for understanding the development of individualized wear patterns on athletic shoes. This presentation will also discuss the development of individual characteristic wear patterns over the course of the study for each of the two athletic shoes examined in this experiment.

In order to ground forensic science in data-driven methodology, it is necessary to assemble reference databases that can be used to benchmark new methods. The longitudinal shoe outsole database hosted on the Center for Statistical Applications in Forensic Evidence (CSAFE) data portal contains images of 160 pairs of athletic shoes, in two styles and four sizes. Participants wore the shoes for at least 10,000 steps per week, and images of the shoes were taken at five-week intervals, producing five observation points for each pair of shoes. At each time point, shoes were imaged using pressure-mat scans, high-resolution photography, 2D digital scanning, 3D scanning, film/dust prints, paper/dust prints, and vinyl/dust prints intended to simulate crime scene prints. For a subset of the shoes, randomly acquired characteristics have been marked and identified by the Israeli Police.

This database, which is freely available to the public, provides a curated data set for examination of Randomly Acquired Characteristic (RAC) development, individual wear pattern characterization, and benchmarking of statistical methods for matching outsole prints. As there are many replicates of each size/style combination in the data set, it is possible to assess within-style individual characteristics and examine how those characteristics develop over time. In addition, as shoes have been imaged using multiple methods, with replicates of each method at each timepoint, it is possible to examine the variability of each collection method and establish the strengths and weaknesses of each method. The database is structured to allow for the download of query-filtered subsets of the images and accompanying metadata. Users can also preview images and 3D surface files using the graphical database interface. Bulk downloads of the full dataset, which is approximately 1.4 TB, will also be available.

This presentation will include a live demonstration of the database and an example of the development of wear over time for one pair of shoes, as shown using several 2D data collection methods.

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#### Database, Outsole, Wear

### E59 Paternity Testing and Next Generation Sequencing: New Approaches to Detecting Illegal Bird Trading

Ana Luiza Queiroz, BA\*, Federal University of Minas Gerais, BELO HORIZONTE, Minas Gerais 31270-901, BRAZIL; Wander U. Mesquita, BSc, Federal University of Minas Gerais, Belo Horizonte 31270-901, BRAZIL; Adriana H. Pereira, MSc, Federal University of Minas Gerais, Belo Horizonte 31270-901, BRAZIL; Susanne Facchin, PhD, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais 31270-901, BRAZIL; Sandra Ludwig, PhD, Federal University of Minas Gerais, Belo Horizonte 31270-901, BRAZIL; Nazaré L. Abreu, MSc, Federal University of Minas Gerais, Belo Horizonte 31270-901, BRAZIL; Isabella Pena, PhD, Federal University of Minas Gerais, Belo Horizonte 31270-901, BRAZIL; Evanguedes Kalapothakis, PhD, Federal University of Minas Gerais, Belo Horizonte 31270-901, BRAZIL

**Learning Overview:** After attending this presentation, attendees will understand important aspects about wildlife trafficking in one of the most biodiverse countries, including its regulation, problems with the current form of identification of legal specimens, and new approaches using DNA to detect illegal bird trade.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing modern techniques applicable to wildlife forensics, especially in regard to illegal bird trafficking.

Birds born in captivity are identified by a small, individually numbered metal tag attached to the leg. However, despite many regulatory standards set for this identification, frauds are frequent and often hard to detect by conventional forensic methods. Paternity tests using microsatellites (Short Tandem Repeats in the DNA) presents an alternative for these cases.

Researchers from the Laboratory of Biotechnology and Molecular Markers developed a standardized paternity test for the blue-fronted Amazon (*Amazona aestiva*) using traditional methodology (Polymerase Chain Reactions (PCRs) followed by analysis of amplified fragments on a 3130 Genetic Analyzer with an estimated cost per sample of approximately USD \$25.00) based on eight loci. This methodology, later employed in a joint inspection with the Brazilian environmental agency, successfully identified fraudulent bird trade, resulting in the shutdown of the breeding sites.

Building on the success of this approach, the same research group then focused on the development of a paternity test for *Saltator similis* (Green-winged Saltator) and *Sporophila maximiliani* (Great-billed Seed-finch), both listed as among the most trafficked species in Brazil.<sup>1</sup> This time, in order to develop a more cost-, time-, and labor-effective methodology, Next-Generation Sequencing (NGS) was utilized, which allowed the manipulation of 384 samples in a single run, representing a cost per sample of approximately USD \$8.00. With this technique, microsatellites on contigs were found using msatcommander and the selected ones had their primers synthesized, PCR reactions standardized, and tested for their degree of polymorphism.<sup>2</sup>

For *Sporophila maximiliani*, ten selected loci were already tested in different populations and proved to be efficient in successfully detecting paternity. For *Saltator similis*, experiments are in the final stages and show promising results.

In summary, paternity testing is a promising technique to help enforcement agencies fight illegal bird trade. The use of NGS considerably facilitates this process, saving time and reducing costs.

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1. Destro, G.F.G. et al. Efforts to Combat Wild Animals Trafficking in Brazil. In: (Ed.). *Biodiversity Enrichment in a Diverse World*. INTECH Open Access Publisher, 2012. p.518.
2. Faircloth B.C. 2008. msatcommander: Detection of Microsatellite Repeat Arrays and Automated, Locus-Specific Primer Design. *Molecular Ecology Resources*, 8: 92–94.

#### Wildlife Traffic, Forensic Genetics, Next Generation Sequencing



### E60 Death Due to Unintentional Dichloromethane Inhalation: A Case Report and Cautionary Tale

Anita Roman Hasert, BS\*, Charleston County Coroner's Office, North Charleston, SC 29405; Demi B. Garvin, PharmD, Forensic Science Network, Columbia, SC 29201; Angelina I. Phillips, MD, MUSC, Charleston, SC 29425

**Learning Overview:** After attending this presentation, attendees will understand the safe and thorough medicolegal death investigation practices needed to: (1) interview those in contact with the victim prior to death; (2) review the victim's social circumstances and medical records; and (3) ensure effective coordination and collaboration with emergency medical services, fire, law enforcement, and Hazardous Materials (HAZMAT). Attendees will also understand specific challenges associated with the scene evaluation, evidence collection, body transport, autopsy protocol, and toxicology testing in a case of fatal hydrocarbon intoxication.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing fatal Dichloromethane (DCM, methylene chloride) exposure and the vital medicolegal death investigation procedures needed to ensure a safe and accurate evaluation of these fatalities.

The true epidemiology of hydrocarbon exposure, morbidity, and death is difficult to determine from available data sources. DCM is a halogenated hydrocarbon commonly found in paint removers, cleansers, degreasers, and aerosol propellants. Reported intoxications from DCM have occurred from inhalation, dermal, ocular, and/or oral exposure. Deaths resulting from unintentional methylene chloride inhalation appear to be relatively uncommon. A colorless, non-flammable liquid with sweet, chloroform-like odor, DCM can rapidly induce general anesthesia when inhaled.

Acute toxic effects may begin immediately upon inhalation with the development of confusion, dizziness, shortness of breath, numbness, nausea, vomiting, and fatigue. Prolonged exposure may lead to lethargy, unconsciousness, and death. The organs primarily affected by DCM are the liver and brain, although an association with cardiovascular disease has also been reported. Unlike other hydrocarbon compounds, DCM is metabolized by liver P450 mixed-function oxidase to carbon monoxide. As a result, significant, delayed, and prolonged carboxyhemoglobinemia has been described.<sup>1-3</sup>

The decedent was a 31-year-old Caucasian male found unresponsive on the floor inside the walk-in refrigerator of his cold brew coffee business. Emergency Medical Services (EMS) were notified and the victim was pronounced dead at the scene. It was determined that he had collapsed while applying a liquid, sprayable paint stripper product containing dichloromethane and methanol. On scene, EMS personnel described the presence of existing and pungent chemical fumes. A HAZMAT team responded to detect, contain, and remove the material, monitor scene safety, and decontaminate the decedent prior to transport and autopsy.

This presentation will include discussion of the history, scene, practices, and personal protective equipment used by the victim, as well as the challenges associated with safe and effective evidence collection in presumed toxic exposures. Pathology and toxicology findings specific to this case will be presented in the context of DCM fatalities previously published in the peer-reviewed literature. Education of the forensic community and the consumer regarding the proper use of hydrocarbon products and their potential hazards is critical to the prevention of toxic and perhaps fatal exposures. Coordination and collaboration with multiple agencies will be necessary for the safety of those involved as well as the accurate determination of cause and manner of death.

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1. S. Dhillon, R. Von Burg. Toxicology Update-Methylene Chloride. *Journal of Applied Toxicology*. 1995, 15(4), 329-335.
2. G. Fechner, Ortmann, A. Du Chesne, H. Köhler. Fatal Intoxication Due to Excessive Dichloromethane Inhalation. *Forensic Science International*. 2001, 122, 69-72.
3. David D. Gummin. Hydrocarbons. In: *Goldfrank's Toxicologic Emergencies*, 10th Edition. Ed.: Robert S. Hoffman et al., McGraw Hill Education, New York, NY, 1334-1345.

#### Paint Stripper, Dichloromethane, Inhalation





## E61 Artificial Intelligence for Homicide Investigations

Jiaqi M. Lu, MS\*, University of New Haven, West Haven, CT 06516; Xuanfeng Fang, MSc, University of New Haven, West Haven, CT 06516; Elaine M. Pagliaro, JD, University of New Haven, West Haven, CT 06516; Henry C. Lee, PhD, University of New Haven, West Haven, CT 06516

**Learning Overview:** The goal of this presentation is to introduce a different approach to performing a forensic investigation that could be assisted by data technology and Artificial Intelligence (AI) and to demonstrate the capacity of these emerging technologies and how they may benefit our daily work.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by utilizing and developing the necessary data technologies to increase the accuracy and efficiency in forensic investigation.

AI is the simulation of human intelligence performed by the computer system.<sup>1</sup> AI research is becoming more and more popular in many professional fields around the world, including finance, health care, industry, education, transportation, smart city management, and many other fields. Research has indicated that the primary feature of AI, learning and problem solving, could also assist in the criminal investigation as well.<sup>2</sup> Although there are many data models and prototype AI systems already developed and employed by the law enforcement community, AI is still an emerging technology for forensic investigation fields. This study attempts to evaluate the potential of AI in forensic investigation and to develop an approach that facilitates an effective investigation through the application of AI.

The proposed approach is built on data and algorithms, and this study concentrated on developing the forensic investigation application through available multidimensional data and efficient algorithms. As a part of computer technology, AI has many unique features in addition to characteristics of the contemporary computer system, including high-performance and accuracy in processing, high capacity in data storage, and high scalability and distribution in network.<sup>3</sup> This research specifically identified the features of AI technology that may assist in the forensic investigation by its particular advantages, especially machine learning, as the most important aspect of AI technology would provide investigators with the data-driven predictions and decisions learning from previous cases. The different analytical models of AI to increase the certainty and efficiency in forensic investigation will be introduced. The models simulate a human's perception to enhance the data process, which image recognition and natural language process would help homicide investigators retrieve the critical information from large-scale of data within minimal time. The analytical models also provide the investigators with crucial patterns by pattern recognition, anomaly detection, clustering, classification, and regression. The linkage with individual, evidence, time, location, pattern, and fact in a case will be developed; associate related or serial cases; eliminate the irrelevant individual and evidence; present the case information with graphical depiction; identify and profile the possible suspect; and develop many other investigative leads from data mining by examining correlations, associations, and factors.<sup>4</sup> Two serial homicide cases will be utilized to demonstrate the process of data collection, data integration, the unstructured data transformation, analysis and reasoning, evaluation and verification, and reconstruction through the application of AI. Details of each logic reasoning and specific data model will be explained as well.

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1. Winston, Patrick Henry. *Artificial Intelligence*. Addison-Wesley, 1999.
2. Russell, Stuart J., and Peter Norvig. *Artificial Intelligence: A Modern Approach*. Harlow, Essex: Pearson, 2014.
3. Charniak, Eugene, and Drew McDermott. *Introduction to Artificial Intelligence*. Reading, MA: Addison-Wesley, 1991.
4. Lee, Henry C., Timothy Palmbach, and Marilyn T. Miller. *Henry Lee's Crime Scene Handbook*. Amsterdam: Elsevier/Academic Press, 2007.

### Artificial Intelligence, Homicide Investigation, Data Mining



### E62 Complex Suicides and Where to Find Them: Disclosing the Mystery

*Giuseppe Bertozzi, MD\**, Department of Forensic Pathology, Foggia 71121, ITALY; *Francesca Maglietta, MD\**, Department of Forensic Pathology, Foggia 71122, ITALY; *Mauro A. Ciavarella*, University of Foggia, Forensic Department, Foggia 71121, ITALY; *Carmela Fiore, MD*, Ospedale “G. Tatarella,” Cerignola, Foggia 71100, ITALY; *Irene Riezzo, MD, PhD*, University of Foggia, Osp D’Avanzo, Foggia 71100, ITALY; *Pietrantonio Ricci, MD, PhD*, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY; *Francesco Sessa, MS*, Department of Forensic Pathology, University of Foggia, Foggia 71100, ITALY

**Learning Overview:** The goal of this presentation is to discuss the wide spectrum of methods employed to commit suicide, including complex suicides, and how a methodological, multidisciplinary approach is mandatory to define the cause of death and serve justice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping pathologists with the management of particular cases, such as complex suicides, and also to show the importance of a methodological multidisciplinary approach to support investigations and find the solution.

Suicide is a serious widespread health problem; according to the World Health Organization data, suicide completion accounts for one death every 40 seconds. Complex suicides deal with more than one method employed to commit suicide, accounting for only 1.5%–5% of all completed suicides. These cases represent a significant challenge for the forensic team, particularly in the definition of the cause of death and the reconstruction of the event. Four cases between 2010–2017 were analyzed by the Department of Forensic Sciences of the University of Foggia. To solve these cases, a multidisciplinary approach was needed and included: a careful crime scene investigation, a complete victim medical history analysis, a meticulous autopsy, with histological and immunohistochemical examinations, and both toxicological and genetic support.

The first case dealt with a 38-year-old man who was found lifeless in the woods with multiple adhesive band loops, obstructing his nostrils and mouth, and two gunshot wounds at the right temporal region and left thoracic side. A gun was located a few meters from the car.

The second case concerned a 32-year-old man found dying in his own car at a highway rest area. He had a deep neck wound, which exposed the underlying hemorrhagic muscular tissues and the laryngeal cartilages. This wound was inscribed in a complex of superficial slashed wounds. The left wrist was also slashed.

Another case dealt with a 76-year-old woman found lifeless in her house, hanging from a scarf wrapped around her neck, in the storage room with multiple superficial and parallel slashed wounds to the flexor surface of both forearms. In her bedroom, a white cotton sheet was found, severely blood stained with a kitchen knife on it.

Last, at a beach resort, a 46-year-old male body was found hanged. A hangman’s noose was made of his car safety belts tied together and was attached to the air conditioning fan. On this fan, a blood-stained knife was also found. His van had a tube connected from the muffler to the right-side window.

In all of these cases, only the mindful integration of all evidence can provide the most reliable reconstruction of the lethal chain of events leading to suicidal choices: all were complex suicides.

To complete the analysis of these cases, given the recent interest in the relationship between polymorphisms in the promoter region of the serotonin transporter gene (SLC6A4 gene) and psychiatric disturbances, this region was tested. In particular, the presence of one or two short alleles of 5-HTTLPR was associated with anxiety-related personality traits and psychiatric disturbances, such as depression. Genetic tests identified the hidden line that linked each case to the others: all subjects were homozygote for short variant.

#### Complex Suicides, Multidisciplinary Approach, 5-HTTLPR Short Allele



### E63 An Eight-Year Retrospective Study on Suicides in Washington, DC

*Breanna M. Cuchara, MFS\*, Manassas, VA 20110; Francisco J. Diaz, MD, Office of the Medical Examiner, Washington, DC 20024*

**Learning Overview:** After attending this presentation, attendees will understand how beneficial it is to perform toxicological analyses on every suicide and monitor the drug trends in suicides, given the current opioid crisis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing evidence that supports a need for states to increase the funding for medical examiners' and coroners' offices. With this increase in aid, the trends in drugs among suicides can be analyzed and new prevention programs can be created by other health agencies.

Suicide is the tenth-leading cause of death in the United States and it is steadily increasing.<sup>1</sup> More than 44,000 people commit suicide every year.<sup>1</sup> When a suspected suicide occurs, the medical examiner, per statute, has jurisdiction over the decedent. A forensic pathologist must consider many factors when ruling the death a suicide, including if the decedent has had suicidal ideations or attempts before, diagnosis of a mental illness, and the amount of drug(s) present in the toxicology report, all of which must be considered before he or she can rule the death a suicide.<sup>2</sup> It is also important to understand the drug quantity and if it had been taken recently or chronically before a suicide. Each of these factors were examined and analyzed during this review.

This study analyzed toxicology, autopsy, and investigative reports for trends within this population. Between 2009 and 2016, the mortality rate for suicides in Washington, DC, had decreased from 8.8 to 6.5 per 100,000 people. Interestingly, the mortality rate was at its highest in 2014 with 10.5 per 100,000 people. When compared to different states, the population size of Washington, DC, was taken into account.

It was found that 394 decedents committed suicide, most commonly by hanging (31.2%), firearms (20.3%), and drug intoxication (15.7%). The majority of decedents were Caucasian males that resided in Washington, DC, between the ages of 20–29 years old. Interestingly, the toxicology trend showed either no drugs (33.7%), ethanol (26.4%), or opioids (14.9%) most frequently detected. Using the test of two proportions, data showed that decedents between the age of 30–39 years old were significantly more likely to have ethanol recognized in their system versus the rest of the population (P value=0.0251). Also, opioids were most commonly found in decedents ages 70–79 years old (P value=0.00398). This study analyzed the method of suicides compared to the month in which this event occurred, presence or absence of suicide note(s), suicide by firearms, the anatomic location of the injury, and more.

This research provides statistical data for public health officials when confronting the issue of suicide. It will help officials move forward with creating outreach programs to specific areas of the community that are at risk for suicide attempts or ideations. The hope is to encourage other offices to perform toxicological analysis on every suicide because drugs play a significant role in behavior. Understanding the significances of prescription or illicit drug use and the effects on behavior will bring health agencies one step closer to decreasing suicide.

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2. What Is a Medical Examiner? *How to Become a Crime Scene Investigator*. Accessed February 21, 2018.

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#### Suicide, Prevention, Mortality Rate



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### E64 Drowning in the District: A Ten-Year Retrospective Review of Cases in the Nation's Capital

*Daniel J. Morgan, MS\*, Washington, DC 20024*

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**Learning Overview:** The goals of this presentation are to discuss: (1) the epidemiological profile of District of Columbia drowning cases, (2) common pre-drowning activities, (3) challenges in determining the manner of death when a body is found in water, and (4) recommended investigative protocols.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding to the body of knowledge of medicolegal investigation of drownings in natural and man-made waterways.

The District of Columbia has a resident population approaching 700,000 and a daily surge in population of workers and tourists. The District of Columbia also has natural and man-made waterways that are highly accessible, yet drowning is a rare phenomenon. For this study, the Office of the Chief Medical Examiner's case management system was searched for the keyword "drowning" in the cause-of-death statements for all accepted cases from 2007-2016. Of the approximately 12,000 accepted cases in this ten-year period, only 100 deaths were attributed to drowning. The results were further filtered by "injury address" to identify deaths occurring in waterways, and 49 cases were identified. Finally, case files were reviewed in order to identify the epidemiological profiles of the decedents and to obtain descriptions of their pre-drowning activities.

In this study, all decedents were identified. The vast majority of decedents were male, which is consistent with published literature. The racial breakdown was as follows: 49% Black, 33% White, 12% Asian, 4% Hispanic, and 2% Other. The percentage of Asian decedents was overrepresented in drowning cases as compared to all other types of medical examiner cases. The age range for decedents was 11 to 76 years with a mean of 45 years old.

Drownings occurred in rivers in 78% of cases, man-made waterways in 16%, and creeks in 6%. The manner of death was Undetermined in 43%, Accident in 31%, and Suicide in 26%. This study may have a higher percentage of Undetermined drownings since the pre-drowning activities were often difficult to identify as the decedents' entry into the water was unwitnessed in 76% of the cases. In the witnessed cases, decedents were involved in recreational activity, lived on/near the water, or intentionally entered the water to commit suicide.

In addition to the challenges of identifying a decomposed "floater," this presentation will discuss two equivocal cases that illustrate the challenges of determining the manner of death. This presentation will conclude with a list of recommended protocols for the medicolegal investigation of bodies found in water.

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#### Investigation, Drowning, Natural Waterways



### E65 A Killer in the Hospital Wards: Murders Committed by the Criminal Administration of Heparin

*Luigi Papi, University of Pisa, Institute of Legal Medicine, Pisa 56100, ITALY; Federica Gori, MD, University of Pisa, Pisa 56100, ITALY; Chiara Toni, MD, Pisa, ITALY; Sara Turco\*, Istituto di Medicina Legale, Pisa 56126, ITALY*

**Learning Overview:** The goal of this presentation is to highlight the importance of a multidisciplinary approach in the police investigation of a complex case of murders in a hospital due to criminal heparin administration. Autoptic examination, histopathological, immunohistochemical, and toxicological analysis together with a meticulous analysis of the clinical reports and Postmortem Computed Tomography (PMCT) revealed 12 cases of “natural death” to be murders due to the voluntary administration of heparin.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the great importance of a multidisciplinary investigation in a nearly unique case of multiple murders due to the criminal administration of heparin by a health care worker.

In May 2015, investigations began from a confidential report to the police, related to the suspicious death of two patients admitted to the intensive care unit of a small-town public hospital. Both cases presented with sudden and unexplained hemorrhagic episodes associated with the evidence of very high levels of heparinemia.

Following this report, a police investigation began. At first, police identified ten cases of similar sudden deaths, all occurring between 2014 and 2015. In all of these cases, sudden death followed hemorrhagic events and was associated with a complete alteration of the coagulation parameters. All the hemorrhagic events were not related to pathologies, either of the therapy administered at home or during the hospital stay.

After May 2015, two further deaths occurred. The last, similar to the other deaths, was characterized by hemorrhagic events and very high levels of heparin in the blood immediately after a surgical procedure for femoral fracture and occurred in September 2015. Inspection of the hospital room revealed an empty bottle of heparin in the garbage bin. Then, another case was discovered: a patient who was in the same room as the first patient of the series. He survived but showed an unexplained alteration of coagulation values. Investigations revealed 13 suspicious cases, 12 deaths, and 1 survivor.

In March 2016, a nurse who worked in that hospital was implicated for heparin administration. The forensic investigation began in March 2016, with a team comprised of a forensic medicine expert, a hematologist, and an anesthetist. Autopsies were performed on eight exhumed cadavers; the other six patients had been cremated. Analyses included a total body-computed tomography and toxicological and histological examinations. The latter was performed using traditional staining with trichrome. The samples of major interest were further examined with an immunohistochemistry analysis based on a glycophorin-test, which is recognized as a reliable test to reveal erythrocytes and the precursors in decomposed tissues.

Despite the advanced state of decomposition, autoptic examination confirmed macroscopic findings of hemorrhagic events described in medical records, while immunohistochemical analysis confirmed the presence of blood in the tissues. The forensic analysis of the case was also based on the thorough examination of the clinical reports, in order to assess the coagulation parameters and therapies.

Further investigation regarding all the patients deceased in that unit from 2014 to 2017 pointed to ten other suspected cases; among these, two were strongly suggestive of voluntary heparin administration and, in one case, the death was related to the combination of heparin and insulin administration.

Worldwide, there is only one other known case of multiple murders due to voluntary administration of heparin, which occurred in the Czech Republic with seven deaths, while in this case, forensic analysis performed with the accurate examination of clinical reports, PMCT, autoptic examination, and histopathological and toxicological evaluation helped reveal the violent death of 12 patients and the death threat of another. The nurse is now on trial, charged with first degree murder. The scientific proof will be fundamental in court.

#### **Heparin, Coagulation, Homicide**



### **E66 Down the Rabbit Hole: The Functional Paralysis of a Medical Examiner Facility by Contaminated Palo Mayombe Artifacts**

*Brett E. Harding, MBA\*, District 5 Medical Examiner's Office, Leesburg, FL 34748; Kyle Shaw, MBBS, District 5 Medical Examiner's Office, Leesburg, FL 34748; Barbara C. Wolf, MD, District 5 Medical Examiner's Office, Leesburg, FL 34748*

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**Learning Overview:** After attending this presentation, attendees will understand: (1) the dangers of elemental mercury facility contamination, (2) the potential danger of Palo Mayombe artifacts and the potential risks to facility and staff, and (3) the multidisciplinary methods utilized to return facilities to functional operations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the dangers associated with Palo Mayombe artifacts and elemental mercury.

The efficient daily operation of a medical examiner/coroner facility is an integral part of the criminal justice system and is essential for the timely service of the citizens who are served. The operations are usually dependent on predictable factors, such as employee staffing, case load, equipment operations, etc. These institutions are expected to maintain a functional operational status through all types of adversary. Protocols address the possible interruption of utility services to help assure the continued operation. However, unforeseen factors or situations arise that may paralyze the successful daily operations of the usually "smooth running" institution.

Contamination hazards within a medical examiner/coroner facility are usually confined to bloodborne pathogens associated with decedents' bodies. These pathogens are generally well understood, and safety procedures have been universally instituted. These biological hazards do not routinely affect the general operation of a medical examiner's office.

Chemical contamination can also be encountered when removing decedents from hazardous environments. Liquids, such as diesel fuel, gasoline, and non-petroleum-related chemicals, can be neutralized or semi-neutralized after the body is removed from the hazardous environment but before transport to the institutional facility for examination.

Gases such as carbon monoxide, cyanide, and methane are usually contained within the environment itself and do not routinely present a threat from "off gassing" once brought into a medical examiner/coroner facility. Potassium and sodium cyanide, often used in jewelry cleaning, may be of particular concern because of the rapidity with which it kills. These effects can be limited to the scene of death and may not pose a risk to operational effectiveness.

Bio-terrorism utilizing bacterial agents, such as anthrax, can also shut down the functional effectiveness of a facility. Unfortunately, in the short term, a false bacterial agent can be as effective as the real bacteria until its authenticity can be verified.

Radiation exposure is a potential hazard but is often limited to the medical therapies within the body itself. These radioactive dangers are limited to the staff performing the internal examination of the body and not to the functional operation of the facility.

Elemental mercury is one of the most common and hazardous contaminants in health care and biomedical research facilities. Its neurological effects are a known danger to individuals who suffer a significant exposure to this toxic "liquid metal." The inadvertent spilling of elemental mercury is the most common method for human exposure. The "Mad as a Hatter" neurologic effects are often a result of chronic exposure from unrecognized spilled mercury and may result in significant medical treatment.

Elemental mercury contamination of human remains, and other objects associated with Palo Mayombe religious practices is an infrequent but real danger encountered by medical examiner's facilities in Florida. The mishandling of such remains may result in not only the interruption of medical examiner services, but substantial monetary costs associated with decontamination and medical evaluation and treatment of exposed staff.

This presentation details the case of an acute elemental mercury contamination within a medical examiner facility. The multidisciplinary methods that are used to continue daily operations, the restoration of functional facility operations, the medical evaluation of staff, and the potential for the establishment of a universal contamination control plan will be highlighted.

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#### **Elemental Mercury, Palo Mayombe, Contamination**



### E67 Look in the Freezer—The Story of a Murder: A Case Report From Portugal

*João Manata, MSc\*, Instituto Nacional de Medicina Legal CF, IP, Coimbra, PORTUGAL; Diogo J. Calçada, MSc, Instituto Nacional de Medicina Legal CF, Coimbra 3000, PORTUGAL; Claudia Marques, MD, Largo Se Nova, Coimbra 3000, PORTUGAL*

**Learning Overview:** After attending this presentation, attendees will understand a case of a homicide in Portugal in which a corpse was found inside a freezer, requiring an extensive forensic investigation until it was resolved, and the murderer arrested.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that postmortem freezing of a corpse is a rare method of concealing a homicide, leaving behind both macroscopic and histological findings related to the mechanics of freezing and thawing, helping to establish the precise cause and manner of death and to distinguish between postmortem artifacts and death by hypothermia.

This study presents a case of an elderly woman, missing for two days, who was found inside a freezer in her kitchen, which was being searched by authorities and friends. Over the freezer, there were no signs of disorder. The corpse was completely frozen, in a supine position, semi-flexed, with her head on a step inside the household appliance. She presented several bruises on the head and upper limbs and abrasions on both lips, on the right upper limb and on the lower left limb. The victim's head and mouth were wrapped in transparent tape and, next to the head, was a lady's handkerchief with "supposedly blood-stained patches." The crime scene investigation did not find signs of a break-in, although there were signs of robbery. According to neighbors, the victim was a belligerent person.

Before the necropsy examination, even though the corpse had been thawed for about two days, it was still partially frozen. The forensic autopsy confirmed the presence of multiple bruises scattered on the body surface, mainly on the scalp and face (translated at external and internal habits), and also on the dorsal aspects of the forearms and the right hand. These findings were compatible with a physical confrontation between the victim and the offender, as the lesions located on the forearms and the hand were suggestive of defensive lesions. From the facial lesions, the blunt wounds and the bruises located at the level of the perioral region and oral cavity were emphasized, with bloody infiltration of the perioral muscular plane and intrinsic muscles of the tongue. Such findings were consistent with a mechanism of smothering. The examination of cervical structures identified a fracture of the base of the right lower horn of the thyroid cartilage, with underlying blood infiltration, that may represent a sign of an extrinsic neck compression. Although the organs, soft tissues, and some biological fluids were still partially frozen, the smothering mechanism was reinforced by the presence of non-specific signs of asphyxial nature, namely pulmonary edema, exuberant visceral congestion, and prominent and diffuse alveolar distention. Diptera eggs were found inside the oral cavity, suggesting that the corpse remained for some time outside the freezer before being placed inside the household appliance. The genital exam performed in the autopsy room showed findings consistent with recent vaginal sexual practices. It should be noted that no macro or microscopic signs consistent with a hypothermic death were observed. In turn, the toxicological tests did not detect the presence of acetone, a compound that, when at high levels, represents a toxicological finding highly indicative of hypothermia. There were no histological findings consistent with artifacts from freezing.

This case report highlights the importance of a thorough forensic investigation, including police investigation and a complete autopsy, in order to determine the manner of death and to define the real cause of death.

#### Smothering, Hypothermia, Homicide



### E68 A Practical Classification for Spree Killers

Mark E. Safarik, MS\*, *Forensic Behavioral Services International, Fredericksburg, VA 22408*; Katherine Ramsland, PhD\*, *DeSales University, Center Valley, PA 18034*

**Learning Overview:** After attending this presentation, attendees will be familiar with a classification system for spree killers that includes subtypes for which it is possible to develop a strategy of identification and capture.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating to the legal and investigative community the need to restore and hone the concept of a spree killer toward the development of tactics for apprehending them and protecting their potential targets.

Multicides have traditionally been categorized as double, triple, mass, serial and spree.<sup>1</sup> Mass and serial have been further divided into subcategories.<sup>2</sup> However, spree killing, which involves the killing of at least three persons at two or more locations in a close timeframe, due to a precipitating incident that fuels the urge to kill, remains a unified but poorly defined concept.<sup>3</sup> The Federal Bureau of Investigation (FBI) eliminated this term from its discussions of multicide in 2005, citing its lack of utility for law enforcement.<sup>4</sup> However, an examination of 112 incidents involving 134 spree killers from 26 countries reveals that not only is there enough diversity among spree killers to form classifications similar to those devised for mass and serial, but also that two spree subtypes offer distinct utility for identification, tracking, and warning potential targets.

This makes the designation of spree killer unique, in that law enforcement personnel who are trained in what to look for can respond efficiently to identify and stop certain types. The behaviors and motives for spree killers align in six categories, at least three of which can be divided into subcategories. Some sprees are random, and some occur in a timeframe or set of locations too tight for an effective response. However, many have identifiable targets, due to grudges or a mission, and some incidents last long enough for law enforcement to mobilize and predict where the killer will likely go. Examples are Christopher Dorner and Dwight Lamon Jones, who left behavioral tracks that served as guides for future targets.<sup>5,6</sup> The killers' agendas were clear and required several days to achieve.

Forty-six of the 112 incidents (41%) are in one of two categories for which law enforcement can form a viable strategy. This percentage supports the need to research spree killers as a distinct type of multicide, standardize the definition, identify categories and subcategories, and provide tools for response strategy. Since the law enforcement response to a spree killer is different than for a mass murderer or serial killer, for those cases for which there is sufficient time to identify potential targets, the data from research on spree killers can be used toward honing an effective response. In addition, spree killer subcategories will inspire more research in criminology for improved comprehension of these killers' diverse triggers and for improved intervention.

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#### Spree Killer, Multicide, Rampage





### **E69 Pozzetto: Inside Behavioral Mazes**

*Edwin O. Olaya Molina, BA\*, Bogotá 111321, COLOMBIA*

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**Learning Overview:** After attending this presentation, attendees will be familiar with one of the most violent episodes in the history of Colombia, the characteristics of the case, and some of the results that emerged from the discussion with the current knowledge of the criminal investigation and forensic sciences.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the status of a criminal investigation in the 1980s in Colombia and how the research activities in this specific case searched for an explanation for what happened and focused on other aspects.

In Colombia, there have been thousands of massacres. Innocent people have suffered directly or indirectly from guerrillas, paramilitaries, drug traffic, politics, and extreme brutality. But after many years, an atypical case in Colombia remains in the memory of many.

The first week of December 1986, one man, Campo Elias Delgado Morales, committed one of the worst crimes that has been registered in Colombia. In a 24-hour period in three different places, armed with a knife and a gun, Morales killed 28 people. He stabbed and burned his mother, her neighbors were shot, he bound and stabbed his English student and her mother, and, finally, after dinner, he shot at several customers in a restaurant named Pozzetto. After an exchange of gunfire with the police, the murderer died. The case was called the Massacre at Pozzetto.

Today, after reviewing photographs, plans, autopsy reports, and statements pertaining to the case more than 30 years later, some of the circumstances of the event are further explained. Forensic ballistics and bloodstain pattern analysis “spoke” about the circumstances surrounding the death of a murderer; the crime scene analysis and behavioral analysis allowed a glimpse of the strong personal feelings of a murderer and a sexual motivation in some of the crimes. Finally, this work attempts to explain what type of killer Campo Elias Delgado Morales was and the particulars of his actions.

This is a case of mistakes and successes, pointing out the importance of comprehensive investigation to seek answers in the criminal investigation, especially the “Why,” which quite often is overlooked in legal proceedings in Colombia. Also discussed will be the contribution of criminal behavior analysis in this and other cases.

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### **Behavioral Evidence, Criminal Behavior, Murder**



### E70 Gargano Mafia: The Fifth Italian Mafia With an Analysis of 129 Murders

Francesca Maglietta, MD\*, Department of Forensic Pathology, Foggia 71122, ITALY; Mauro A. Ciavarella, University of Foggia, Forensic Department, Foggia 71121, ITALY; Giuseppe Bertozzi, MD, Department of Forensic Pathology, Foggia 71121, ITALY; Giuseppe Davide Albano, MD, Foggia 71121, ITALY; Angelo Montana, MD, University of Catania, Catania, ITALY; Anna Cornacchio, Department of Forensic Pathology, Foggia 71121, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY; Monica Salerno, MD, PhD\*, Department of Forensic Pathology, Foggia 71121, ITALY

**Learning Overview:** The goal of this presentation is to examine the fifth Italian mafia, the “Mafia of Gargano,” the youngest of the famous criminal organizations in Italy, its homicidal mode, type of weapons used in murders, and the typology of action.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the distinctive features of a criminal association in Puglia through the analysis of several murders committed there.

Four principal criminal organizations are known to exist in Italy: “Cosa Nostra” in Sicily, “Camorra” in Campania, “Sacra Corona Unita” (SCU, United Holy Crown) in Puglia, and “Ndrangheta” in Calabria. It is also well known to the Italian authorities that there are several subsidiary independent criminal groups that modeled their organizations after the Mafia. In the Apulian Region the SCU developed: The “Foggia Society” and the “Garganic Mafia.” The Mafia’s historians traced back the origins of “Foggia Society” to January 1979, when the main criminal leader of the area was in a meeting at the Hotel Florio, on the road from Foggia to San Severo. The Godfather of the occasion was Raffaele Cutolo, Neapolitan Mafioso of the “New Organized Camorra.” The Godfather arrived in Foggia and explained the dynamics of organized crime and affiliated the local gangsters. He also explained the Mafia method and created the “Foggia Society.” The “Mafia of Gargano” began their criminal activities in the 1990s with a series of violent murders committed between 1990 and 2002.

This group was initially classified by both the district attorney and local authorities as a group of “familial thugs” of rural origin, but it is now known as a large, notorious organization, recognized in 2009 as a “mafia-like criminal association.” In the last decade, the “Mafia of Gargano” established its power and control in both national and international laundering, human smuggling, illegal distribution of drugs and tobacco, and trafficking in weapons from the east countries by way of the Balkans. Some “Mafia of Gargano” murders are intended to portray the power of a group and its ability to control a territory. On the other hand, murders have the goal of eliminating individuals that may represent an obstacle to the criminal organization’s business. This statistical study analyzed 129 cases of homicides committed by firearms between 2000 and 2015 in the Foggia countryside. In all, 69 incidents occurred in the “Gargano area,” all planned to follow the pattern of attack, and the other 60 incidents happened in suburban areas and around Foggia. These murders were categorized in regard to the type of weaponry and homicidal modality, with particular care taken of the description and classification of the target body regions.

Subsequently, the type of weapon used for the various murders was taken into consideration; this is another significant variable characterizing the classic *modus operandi* of the Gargano Mafia. In 42 out of 69 homicides, the weapon typically used is the rifle, while in the remaining 26 homicides, the weapon used is handgun, whereas in the murders in the Foggia area, the weapon typically used is a handgun, characterizing as many as 47 homicides out of 60. In the Gargano Mafia murders, it is possible to underline that 26 murders exhibited the presence of the *coup de grace*, but all followed the pattern of an ambush. The complete analysis of these homicides sheds some light on a specific “way to kill” that binds and defines the “Gargano Mafia murders, establishing the existence of a canonical procedure of preparation and execution of the criminal events with the primary intent of avoiding an open casket funeral. Predominant is the will to express the strength of the group and its affiliates, the total disregard of rules among members, the recourse to deception to lure the victim, and the involvement of innocent unrelated people—all elements that characterize the “Mafia of Gargano.”

#### Italian Mafia, Criminal Organization, Mafia Murder



### E71 A Closer Look Into Latino Youth Gang Violence: From MS13 to the Latin Kings

*Cliff Akiyama, MPH, MA\*, Akiyama and Associates, LLC, Philadelphia, PA 19143*

**Learning Overview:** The goal of this presentation is to educate attendees about Latino youth gangs and the violence that is seen on a daily basis throughout the United States, in order to keep themselves safe out on the streets.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making attendees more aware of the “signs and symptoms” of Latino youth gang violence, so that they may keep themselves safe while investigating the deaths of these individuals.

One cannot open the newspaper or watch the local news without hearing of another casualty of gang violence. Throughout the country in urban, suburban, and rural communities, gang violence has reached epidemic proportions, according to the Department of Justice, Office of Juvenile Justice and Delinquency Prevention. Furthermore, gangs have been identified in every state, meaning that gangs are no longer just a California problem. Nationwide there are 24,500 gangs with a gang membership of more than 750,000, with the ethnic composition of these gangs including 47% Latino, 31% African American, 13% Caucasian, 7% Asian, and 2% Mixed. In particular, youth gang violence in the Latino community has had a dramatic increase in the past two years. In Los Angeles County, CA, alone, there are currently 750 documented Latino gangs with a gang membership of more than 93,000. Demographics report a gang member average age of 15 years old with a range of 8–22 years of age. Other counties in California and nationwide have seen increases in Latino gangs, especially in rural communities such as York, PA. The Mara Salvatrucha (MS 13), an El Salvadorian gang originating in the Pico-Union area of Los Angeles, CA, since 1983, has seen the largest increase in membership nationwide, with more than 67% in some states. More than 600 gang members were interviewed for this study out in the streets, jails, and juvenile halls in California and Pennsylvania. This study identified eight distinct manifestations of gang violence and nine ethnic differences and similarities among Latino gangs (i.e., definite cultural differences between Latino gangs and various other ethnic gangs, drugs, weaponry, killing over turf/territory, extortion, defacing property/graffiti, and women in gangs).

This study found that Latino gangs are motivated by a state of mind driven by “La Raza,” which translated means “for the race.” It is important to note that La Raza is more of a cultural ideology than a gang-related motto. Latino gangs are extremely territorial and unlike African American gangs where the individual is important, for Latino gangs, it is the gang as a whole that is important and not the individual. To illustrate this point, when Latino gang members go to prison, one of two prison gangs run and controlled the environment depending on the geographic location of the prison: La Eme or Nuestra Familia. La Eme, otherwise known as the Mexican Mafia, is a prison gang originating in California and is considered the leadership arm of all Latino gangs in Southern California. The letter “M” in Spanish is pronounced “eme” and is the 13<sup>th</sup> letter of the alphabet. Consequently, throughout Southern California, Latino gangs will often call themselves by the city or area that they represent, followed by the number “13” to indicate “La Eme” or “Southern” by giving respect to the Mexican Mafia. In Northern California, the Nuestra Familia, which translated means “our family,” is the prison gang that controls every Latino gang north of Fresno, CA, and is often indicated by the number “14,” representing the letter “N” for Nuestra and Northern. It is important to remember that Latino gangs are not just a California problem, although California is where these gangs originated. Rural, suburban, and urban communities across the nation are now seeing an increase in Latino gangs that mimic the California-based Latino gangs, such as the MS 13.

In direct response to the increase in gangs, the state of California, North Carolina, Virginia, and Idaho have passed gang enhancement laws, which increase the penalty if an individual is found to have committed a felony for the benefit, association, or direction of a criminal street gang. Other states, including California, have also filed a civil injunction or “gang injunction” against specific gangs, whereby removing the leadership of the gang and disbanding its members.

The purpose of this presentation is to present timely data on Latino gangs and offer strategies on how to recognize and interpret various tattoos and graffiti associated with these gangs, which could assist the medical examiner/coroner and death investigator in the positive identification of the decedent out in the field and/or in the autopsy room. This presentation will also discuss some of the recent gang-enhancement laws that California, North Carolina, Virginia, and Idaho have in place to help tackle this deadly problem.

#### **Latino Gangs, Gang Enhancement Laws, Youth Violence**



### E72 The Cruel Deaths by Suffocation of 71 Migrants Smuggled From Hungary to Austria: A Tragic Case Study

*Armin A. Farid, DMD\*, Budapest 1067, HUNGARY; Christoph Reisinger, MD, Medizinische Universität Wien, Vienna 1090, AUSTRIA*

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**Learning Overview:** The goal of this presentation is to discuss forensic methods of identification and legal procedures, as well as humanitarian aspects of human smuggling.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by detailing the dangerous, cruel, and inhumane consequences of human smuggling, so common in different parts of the world.

In 2015, Europe became the scene of mass immigration, especially over the so called “Balkan Route” from Turkey to Greece, and from Macedonia, Serbia, and Hungary to Austria and Germany. During this time, smugglers made huge fortunes transporting desperate refugees from the east to the west of Europe. The unscrupulous actions of such smuggling rings not only exploit the immigrants but puts lives in grave danger due to the lack of sufficient means of safe transportation from one location to another.

In August of 2015, a truck was discovered on the roadside of an Austrian motorway, containing the bodies of 71 immigrant men, women, and children who began the journey in Hungary. Only 30 minutes after the truck left its place of origin, the passengers were struggling to breathe due to the crowded conditions and lack of sufficient oxygen. Three hours later, all had died. The cause of death was determined to be suffocation.

Hungarian and Austrian authorities immediately began an investigation that resulted in the arrest of four smugglers. They determined the time of death to have taken place in Hungary where the journey first began. Austrian officials took on the task of identifying the victims, primarily using DNA analysis, while Hungarian law enforcement handled the legal proceedings resulting with the criminal conviction of the smugglers in June of 2018. Considering most immigrant deaths in Europe occur at sea while crossing the Mediterranean from North Africa to Italy and Malta, this large death toll occurring on land could have been avoided and was due to the extreme inhumane conditions, crowding 71 people in the back of a truck with no oxygen.

Despite the call of the prosecution for a life sentence, the four smugglers, one from Afghanistan and three from Bulgarian descent, were convicted and sentenced to only 25 years imprisonment in a court in Central Hungary, with no prospect of earlier release. Altogether, 14 people were involved in this smuggling ring, and the involvement ranged from planning and organization of vehicles for smuggling people to hiring drivers. The four main leaders who held key positions in the ring who were convicted for 25 years consisted of the ring leader, the deputy ring leader, the truck driver, and the driver of the car that followed the truck during this “journey to death.” The other ten people involved were also convicted and sentenced to somewhere between 3 to 12 years imprisonment, depending on their degree of the involvement in this unimaginable tragedy. The international character of this case, involving the two neighboring countries of Austria and Hungary, demonstrates on a small scale how all of Europe is affected by this crisis.

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#### Identification, Smuggling, Suffocation



### E73 A Crucifixion Experiment to Assess Wrist and Forearm Blood Flows as Observed on the Shroud of Turin

*John P. Jackson, PhD\**, Turin Shroud Center of Colorado, Colorado Springs, CO 80922; *Keith E. Propp, PhD\**, Colorado Springs, CO 80936; *Kim M. Look, DDS\**, Colorado Springs, CO 80916; *Rebecca S. Jackson, MBA\**, Colorado Springs, CO 80936

**Learning Overview:** The goal of this presentation is to test the blood flow patterns on the Shroud of Turin with actual crucifixion configurations of a human body.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by utilizing medical forensics, physics, and historical data to probe and provide insight into the practice of crucifixion, using the Shroud of Turin as a possible example of that ancient practice, and by possibly providing help with other forensic problems or having forensic applications to archaeology.

A recent paper reported by Borrini and Garlaschelli concluded from blood flow experiments that the observed wrist/forearm blood flow patterns on the Shroud of Turin are sufficiently inconsistent with the studies so that the Shroud of Turin should be considered to be a probable forgery.<sup>1</sup> However, the conclusion of the scientific experiments and analyses of the same blood flows have reached the opposite conclusion.

In this presentation, the forensic analysis is based on live suspensions on a cross with volunteer subjects, a methodology that was not used by Borrini and Garlaschelli to reach the conclusions.<sup>1</sup> After reviewing and discussing important pertinent historical data and archaeological artifacts related to the practice of crucifixion in the ancient Roman world, this study designed an experimental protocol by which special wrist and foot attachment mechanisms safely and realistically suspend the male subjects on a full-size cross. Professional medical personnel were invited to not only contribute to the experimental protocol and analyses, but also to ensure the medical safety of the subjects. The male subjects were carefully chosen to correspond, as closely as possible, to the physiology depicted by the frontal and dorsal imprints visible on the Shroud of Turin. A comprehensive evaluation was performed of the totality of blood flows found on the Shroud to determine which flows occurred during the alleged crucifixion process and which were of a postmortem nature. The specific crucifixion nailing characteristics and locations represented by the Shroud image were also determined. The subjects were then suspended on the cross according to those determinations. The cross and suspension system were designed to accommodate various positional adjustments of the body as appropriate.

Once the study team and supporting review team were satisfied with the validity of the crucifixion positions of the subjects, blood was deposited externally on the body at the previously determined nail sites. The resulting flow patterns over the simulated, crucified subjects were documented and analyzed. The crucifixion positions of the subjects were likewise documented.

These experiments represent an important utilization of medical forensics, physics, and historical data to probe and provide insight into the practice of crucifixion, using the Shroud of Turin as a possible archaeological depiction of that ancient practice, the details of which are virtually unknown in modern times. Moreover, the techniques that were specifically developed for this study conceivably may be helpful in analyzing certain other forensic problems, as well as in forensic applications to archaeology.

The presentation, using the perspectives from the above disciplines, will discuss how conclusions were obtained that appear to support the hypothesis of Shroud authenticity in some new and unexpected ways.

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**Shroud of Turin, Blood Flow Pattern, Crucifixion**



### E74 Testing Sexual Assault Kits Supports the Principle of “Justice For All”

*Julie L. Valentine, PhD\*, Brigham Young University, Provo, UT 84064; Suzanne Miles, BS\*, Utah Bureau of Forensic Services, Taylorsville, UT 84129; Leslie Miles, DNP, Brigham Young University, Provo, UT 84602; Linda Mabey, DNP, Brigham Young University, Provo, UT 84602*

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**Learning Overview:** After attending this presentation, attendees will understand research data on Sexual Assault Kit (SAK) submission rates and DNA analysis findings that supports the testing of all SAKs to establish justice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by strengthening the justification to test all SAKs with research data from a large, retrospective study.

In most areas of the United States, law enforcement agencies have been the decision-makers regarding the submission of SAKs to crime laboratories for analysis. SAKs not submitted have either been maintained in law enforcement storage or destroyed. Recently, law enforcement agencies have been encouraged to submit previously unsubmitted SAKs for DNA analysis. Victim advocacy groups have encouraged the submission and testing of all SAKs to ensure justice for victims.

This presentation provides research findings from a large, retrospective study on the submission rates of 4,038 SAKs (collected from 2010 to 2016) and subsequent DNA analysis findings. Predictors of SAK submission rates through Generalized Estimating Equation (GEE) logistic regression modeling will be shared. Research data on the apparent subjectivity in the decision-making process to submit SAKs for testing will be discussed. Short Tandem Repeat (STR) and STRs found on the male-specific Y-chromosome (Y-STR) DNA analysis findings of SAKs submitted within 1 month, 1–12 months, and 1 year or more from the date of assault and evidence collection will be provided. No significant differences were found between DNA analysis findings on SAKs submitted within 1 month, 1-12 months, and 1 year or more from the date of assault and evidence collection. This finding supports the submission and testing of all SAKs as those SAKs submitted soon after the crime had similar DNA analysis findings as those kits previously in law enforcement storage. Although DNA analysis findings from SAKs were similar between jurisdictions, the determination of DNA profiles as Combined DNA Index system (CODIS) -eligible profiles was found to have increased variability.

This retrospective study also explored the identification of sexual assault cases in which the DNA analysis findings excluded the identified suspect. It was found that approximately 2% of tested SAKs had DNA analysis findings that excluded the suspect, therefore also establishing justice for the accused. Testing SAKs is often touted as establishing justice for victims, but findings from this study also indicate testing SAKs is necessary to establish justice for both victims and the accused.

At the conclusion of this presentation, attendees will understand research data which supports the submission and testing of all sexual assault kits to support the principle of “Justice For All.”

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**Sexual Assault Kits, DNA, Justice**



### E75 Forensic Doctors and Clinical Forensic Medicine: Should or Could?

*Pierre-Antoine Peyron, MD, Département de Médecine Légale, Montpellier 34295, FRANCE; Elodie Michel, Department of Forensic Medicine, Montpellier 34295; Eric Baccino, MD\*, Hopital Lapeyronie, Montpellier, Cedex 5 34295, FRANCE*

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**Learning Overview:** The goals of this presentation are: (1) to question whether or not forensic doctors have an interest in being involved in Clinical Forensic Medicine (CFM), (2) to evaluate if the appropriate training is obtained, and (3) to assess where forensic nurses fit in this field.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing how the French medicolegal system allows CFM to be integrated into the daily practice of forensic doctors thanks to close collaboration with forensic nurses.

Even if statistics are lacking, there are two reasons to support the general feeling that the number of “traditional” forensic doctors is not increasing: (1) a decline in the autopsy rate due to the rapid development of imaging techniques (so called “virtopsy”) and also to “ethical,” legal, and political opposition, (2) the attractiveness of a much more lucrative private practice for highly trained forensic doctors (i.e., forensic pathologists).

In countries with no Anglo-Saxon forensic tradition, the examination of living victims of violence (mainly “ordinary” violence but also torture) by forensic doctors who are not always specialized in histopathology (but rather in performing autopsies) is developing rapidly, sometimes in association with the examination of people in custody and bodily damage evaluation.

Forensic doctors have an interest in being involved in CFM as the activity related to living victims has a huge potential for development and, consequently, will result in an increasing number of forensic doctors. To be able to practice CFM, forensic doctors should have a basic General Practice (GP) training as well as special training in drug addiction, gynecological examination, child abuse evaluation, basic psychiatric knowledge, etc. Those who specialize only in histopathology would need additional specialized training in CFM.

In some countries facing a lack of availability of forensic doctors and a much higher cost of medical intervention in emergencies, trained and dedicated forensic nurses can provide adequate service in the narrow field of CFM. Since 2011, France has benefited from a new state-funded national medicolegal system, encompassing all aspects of forensic medicine (autopsy, histopathology, CFM).

In this presentation, the French organization in which forensic doctors and forensic nurses collaborate in the best interest of justice, patients, and our professional bodies will be presented and analyzed. The literature data on the organization of CFM worldwide from a medical and nursing point of view will also be discussed.

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#### Clinical Forensic Medicine, Forensic Doctors, Forensic Nurses



### E76 DNA Evidence Collection and Analysis in Groping Sexual Assault Cases

*Julie L. Valentine, PhD\*, Brigham Young University, Provo, UT 84064; Suzanne Miles, BS\*, Utah Bureau of Forensic Services, Taylorsville, UT 84129*

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**Learning Overview:** After attending this presentation, attendees will have better understand the importance of DNA evidence collection in stranger groping assault cases and best practice guidelines for evidence collection in groping sexual assault cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing best practice guidelines in DNA evidence collection in stranger groping sexual assault cases.

Within a state database of 4,038 sexual assault kits linking information from evidence collection to DNA analysis findings, 66 groping cases were identified in Utah. Approximately 250 legal or extralegal variables were coded on each case to explore victim and assault characteristics in stranger groping cases. A summary of key variables on the stranger groping cases will be briefly discussed. Information regarding sexual assault kit submission rates in the groping cases will be provided. The DNA analysis findings on groping cases submitted for testing will be shared and compared to DNA analysis findings on sexual assault kits in which victims had contact with body fluids from assailants.

An interesting case report will be reviewed on the successful identification of a serial groper on a university campus from touch DNA analysis findings. An overview of the Short Tandem Repeat (STR) and Y-chromosomal Short Tandem Repeat (Y-STR) DNA results found in this case will be shared to explore the practice implications on evidence collection and analysis in sexual assault groping cases.

The 66 groping cases found in the Utah database represent a mere 1.6% of collected sexual assault kits from 2010 to 2016. The findings from this study and case report support increasing evidence collection in sexual assault groping cases. To address the need to increase evidence collection in stranger groping cases in Utah, an educational program by medical/nursing forensic examiners and forensic scientists has been developed and presented to multiple law enforcement agencies. Information on the educational program and the statewide Touch DNA Evidence Collection Form will be shared with attendees. Key points from a literature review on touch DNA analysis findings in sexual assault cases will be reviewed. Best practice guidelines based upon study findings and literature review in collecting evidence for DNA analysis in stranger groping cases will be presented.

Additionally, this presentation focuses on exploring the benefits of a strong working relationship between medical/nursing forensic examiners with those processing the biological evidence, forensic scientists, to achieve optimal results in DNA findings in sexual assault and stranger groping cases. Collaboration between medical/nursing forensic examiners and forensic scientists is necessary to establish and achieve best practice guidelines for evidence collection in sexual assault cases.

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#### Groping Cases, DNA, Evidence Collection





### E77 Forensic Nurses in Institutes of Forensic Medicine: An Evaluation of the Zurich Integration Model

*Valeria Hofer\**, University of Zurich, Zuerich CH-8057, SWITZERLAND; *Michael Thali, MD*, Universitat Zurich, Zurich CH-8057, SWITZERLAND; *Rosa M. Martinez, MD*, Universitat Zurich, Zurich CH-8057, SWITZERLAND

**Learning Overview:** After attending this presentation, attendees will have a deeper understanding of a specific role of forensic nurses in the institutes of forensic medicine. It is proposed that a similar model may be worth implementing at other forensic medicine institutes globally.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the added value a forensic nurse brings to the table in terms of documentation and sampling. Forensic investigation, both in the dead and living, is an ever-evolving and growing field, requiring expertise at many levels.

Since the autumn of 2016, the Institute of Forensic Medicine at the University of Zurich, Switzerland, has employed a nurse (Advanced Federal Diploma of Higher Education), who is also a university graduate in Certificate of Advanced Studies (CAS) forensic nursing.

An increased demand for qualified personnel in forensic health care has existed over the past years. As a result, the implementation and application of forensic nursing science is in the pioneering stages at institutes of forensic medicine in Switzerland, and this development has been very exciting thus far. The Institute of Forensic Medicine in Zurich was the first institution in German-speaking parts of Europe to employ and integrate a forensic nurse in daily forensic medicine practice. Accordingly, other institutes of forensic medicine in Switzerland followed this trend and are developing specific training and educational programs for nurses.

After the first year of having a forensic nurse on-site at the Institute of Forensic Medicine in Zürich, the outcomes were evaluated in order to examine: (1) the current situation in terms of service delivery, and (2) the status of practical integration of the forensic nurse employed at the Institute of Forensic Medicine in Zurich. A questionnaire was designed to rate the value of forensic nursing in terms of clinical and postmortem forensic work in the opinion of doctors working in the field. The forensic nurse played an active role in assisting doctors with documentation, photography, obtaining and securing blood, urine, and hair samples of suspects or victims, and negotiation with clinical personnel at medical facilities. The forensic nurse was also utilized at large-scale police checkpoints, where motor vehicle drivers were screened for their fitness and ability to drive, among other forensic tasks. The results conformed to a double hump curve, with two peaks: one with a high and one with a low rating. These findings correlated strongly with the level of exposure to forensic nursing assistance, in the sense that the doctors who regularly attended cases with the forensic nurse were generally more positive about the experience, whereas some forensic medical practitioners who rated forensic nursing negatively had not ventured to conduct case work alongside the forensic nurse in order to directly observe and experience the possibilities of this new inter-professional cooperation. One interpretation has been that the doctors who did not wish to utilize the support of a forensic nurse had been overly influenced to be entirely self-sufficient. The doctors who actively integrated with the forensic nursing service also reported an increase in acquiring practical forensic skills, resulting in a win-win situation.

In conclusion, evaluation of the contribution of forensic nursing to service delivery at an institute of forensic medicine in Switzerland one year after implementation of the service revealed that integration of a forensic nurse into the field of forensic medicine, albeit a complex task, holds great promise.

#### Forensic Nursing, Institutes of Forensic Medicine, Evaluation



### **E78 When the Confirmation Sample Doesn't Match the Profile Entered Into the Combined DNA Index System (CODIS): Troubleshooting the High-Throughput Database Process**

*Devon Hall, MFS, Maryland State Police, Pikesville, MD 21208; Bruce J. Heidebrecht, Maryland State Police, Forensic Sciences Division, Pikesville, MD 21208; Michelle Groves, MS\*, Maryland State Police Forensic Sciences Division, Pikesville, MD 21208; Argiro G. Magers, MS, Pikesville, MD 21208*

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**Learning Overview:** After attending this presentation, attendees will be aware of the need to verify the accuracy of profiles obtained from database samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by alerting scientists to the possibility of a DNA profile being incorrectly associated with a specific database sample and of a strategy created by the Maryland State Police-Forensic Sciences Division (MSP-FSD) to efficiently troubleshoot this issue and correct any identified errors.

A CODIS hit occurred at Maryland's State DNA Index System (SDIS) level between a database sample and a forensic profile from one of the Local DNA Index System (LDIS) laboratories. The LDIS laboratory requested the match be processed for the offender's information. Prior to releasing the offender's information to the requesting agency, MSP-FSD procedure requires any database sample involved in a hit to be tested a second time to confirm that the original profile entered into CODIS was correct. The confirmation testing of this specific sample produced a different profile when compared to the original profile entered into CODIS. This discrepancy led to an immediate investigation and the samples involved were re-tested to obtain the correct DNA profiles. Upon completion, the CODIS hit was re-generated to the correct database sample and the offender's information could then be released to the requesting agency in relation to the CODIS hit.

During the troubleshooting of the original incident, scientists determined the error may have occurred during the initial testing of the database samples and may have been due to a sample punch landing in a different well than expected during the automated processing. It was also determined that this error may be more widespread than initially believed. Scientists determined the troubleshooting would require the investigation of all database samples with duplicate profiles in CODIS. Nearly 23,000 duplicate matches, involving more than 45,000 samples, were researched to verify their accuracy. MSP-FSD scientists developed a method to automate a majority of the research into each of these, therefore reducing the number of samples that needed to be manually researched.

During the troubleshooting process, scientists found that, in addition to the original error detected during the hit confirmation, there were other types of errors occurring in the system that needed to be researched and addressed. These errors were occurring prior to receiving the sample at the laboratory, during the collection process, and while being processed in the laboratory. Procedures were put in place at MSP-FSD to identify and correct all types of past issues that were identified. Procedures also needed to be created and implemented to detect and address any newly identified errors in a timely manner moving forward. Thankfully, none of the identified errors resulted in MSP-FSD missing a CODIS hit; however, this incident did bring to light potential threats that could impact other CODIS laboratories.

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#### **CODIS Hit, Troubleshooting, High-Throughput**



### E79 Rapid DNA Implementation—Experiences From an Accredited Laboratory

*Kristen Naughton, BS, Bode Technology, Lorton, VA 22079; Stephanie R. Sivak, MS\*, Bode Cellmark Forensics, Lorton, VA 22079; Jamia J. Mealy, MS, Bode Technology, Lorton, VA 22079*

**Learning Overview:** After attending this presentation, attendees will: (1) be introduced to Rapid DNA technology and data, (2) be provided with an overview of the standards governing the use of Rapid DNA, (3) learn the challenges of implementing Rapid DNA testing in an accredited DNA laboratory, and (4) learn the importance of setting recommendations for partnered law enforcement agencies to follow to maintain courtroom confidence in DNA testing technology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a blueprint as to how an International Organization for Standardization (ISO) 17025 accredited laboratory can adopt and assist law enforcement agencies to successfully implement rapid DNA technology. This presentation will focus on the importance of implementation recommendations that meet and/or exceed current Quality Assurance Standards to ensure the integrity of DNA testing, even in the hands of non-DNA experts.

Rapid DNA describes the fully automated hands-free process of developing a Combined DNA Index System (CODIS) Core Loci Short Tandem Repeat (STR) profile from a reference sample. Rapid DNA instruments are designed specifically for use by law enforcement in non-laboratory environments, such as booking stations. This “swab in—profile out” technique generates results in just 90 minutes, providing both DNA laboratories and law enforcement with immediate information to aid in investigative leads.

This presentation will delve into the changes that Bode Cellmark, as an ISO 17025 certified laboratory, made to accommodate rapid DNA into current casework workflows. This included the training of DNA analysts in both Rapid DNA instrumentation and modified Rapid DNA analysis methods. A brief overview of the internal validation will show attendees an example validation plan that meets both ISO/International Electrotechnical Commission (IEC) 17025 and the Federal Bureau of Investigation Quality Assurance Standards, including discussion of the new standards expected to be released in early 2019. This presentation will also demonstrate the hurdles of bringing a newly released technology on-line, sample type compatibility with the current Rapid DNA instruments, and a review of challenging type samples that will encompass discussion on the current limitations of Rapid DNA.

With the recent passage of the Rapid DNA Act in August 2017, a move toward expanding the ability of law enforcement to implement Rapid DNA analysis technology and profile upload into the federal database has begun. Commercially available Rapid DNA instruments have been targeted toward law enforcement agencies; however, policies for this new technology have yet to be instituted. Establishing best practices within the forensic community is crucial. State and local crime laboratories inevitably will face similar inquiries about Rapid DNA from the local law enforcement agencies. This presentation provides implementation recommendations for both DNA laboratories and law enforcement agencies regarding appropriate documentation of validation, training, and maintenance of instruments to meet accreditation standards.

Rapid DNA technology is a great advancement for the field of forensics, but the implementation must be a cooperative effort between law enforcement and crime laboratories to ensure that accurate, reliable, and reproducible data is produced and presented in a court of law.

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#### Rapid DNA, Quality Assurance, Law Enforcement



### E80 The Smell of DNA: How Genetics and Fear Influence the Human Scent

*Francesco Sessa, MS\*, Department of Forensic Pathology, University of Foggia, Foggia 71100, ITALY; Marcello Rendine, DBA\*, Department of Forensic Pathology, Foggia 71100, ITALY; Carmela Fiore, MD, Ospedale "G. Tatarella," Cerignola, Foggia 71100, ITALY; Stefania De Simone, MD, Department of Forensic Pathology, Foggia 71122, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY; Irene Riezzo, MD, PhD, University of Foggia, Osp D'Avanzo, Foggia 71100, ITALY*

**Learning Overview:** After attending this presentation, attendees will better understand how genetics and fear can modify the human scent.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an explanation of how genetic characteristics and fear-stressor situations could influence the human scent, creating great difficulties for detection dogs.

Human scent identification is based on a matching-to-sample task in which trained dogs are required to compare a scent sample collected from an object found at a crime scene to that of a suspect. Based on dogs' greater olfactory ability to detect and process odors, this method has been used in forensic investigations to identify the odor of a suspect at a crime scene. These dogs are also used to search for missing persons.

Many studies have shown that human scent can persist and maintain its main chemical features for a significant period of time in a particular place or/and on an object that was manipulated by a subject. For this reason, search and rescue dogs are trained to follow the scent of each person to be located and finally rescued. The results are very surprising; in previous studies, perfect responses were obtained. But, unfortunately, real cases frequently report non-positive results, especially in the recovery of missing people. One of the causes of the failed findings of the missing persons is the so-named "smell of the fear" that could confound the dog, covering the original scent of the missing person.

In specific situations, the stress management represents an important aspect to releasing the confounding scent. Moreover, several studies have provided strong evidence for an association between a polymorphism in the promoter region of the serotonin transporter gene (SLC6A4 gene) and neuroticism, defined as proneness to negative emotionality, including depression and anxiety.

The goal of this experimental study is to define how genetics influences the release of the confounding scent in the particular stressor events that occur in real forensic cases, such as a homicide or missing person. Several volunteers were tested for the 5-HTTLPR polymorphism. Two groups will be defined: ten in the group's homozygotes for the long variant (good management of the stress) and ten in the group's homozygotes for short variant will be enrolled.

A t-shirt was worn for ten minutes under normal circumstances, without a stressor event ( $T_0$ ). Then another t-shirt was worn after a stressor event ( $T_1$ ). All t-shirts were concealed in different places. Subsequently, a trained dog was used to find the t-shirts, with the aim of verifying if the stressor event in the subject with the short variant had changed the released scent. Finally, a mass spectrometry analysis was performed to analyze if the characteristics of the released scent were modified between the two tests ( $T_0$ - $T_1$ ).

After attending this presentation, attendees will have a better comprehension on a direct implication of the stressor events on the forensic investigation. Moreover, it will be illustrated that genetic characteristics could influence the human scent.

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#### DNA, Smell of Fear, Detection Dogs



### E81 A Raman Spectroscopic Method for Semen Identification: Azoospermia

Marisia A. Fikiel, MS\*, State University of New York at Albany, Albany, NY 12222; Igor K. Lednev, PhD, State University of New York at Albany, Albany, NY 12222

**Learning Overview:** After attending this presentation, attendees will understand: (1) the need for a non-destructive and more specific test for human semen, (2) the advantages of using Raman spectroscopy and chemometrics to identify semen, and (3) how sperm contribute to the Raman spectra of semen.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing alternative methods for body fluid identification that are improvements over current methods.

This presentation will focus on semen, a body fluid that is especially important in sexual assault cases, and the validation of an alternative method for its identification. Semen is a special body fluid because it is made of two distinct parts, seminal fluid and sperm. Sperm are the most unique part of semen and, therefore, the presence of sperm is the most reliable method of positive semen identification. However, current semen tests rely on the detection of proteins that are not unique to semen in the seminal fluid because some semen contains no sperm, a condition called azoospermia. The laboratory has been developing a Raman spectroscopic test for the identification of dry traces of body fluids, including semen, for forensic purposes. An automatic software has already been built for differentiating all main body fluids.<sup>1</sup>

The main objective of this study was to evaluate the ability of Raman spectroscopy to identify semen traces in the absence of sperm. This will ensure that the method is compatible, even with semen in disease states. For this purpose, a comparative analysis of Raman spectra of semen, seminal fluid, and sperm samples obtained from several donors was conducted. The spectra of seminal fluid were very similar to that of whole semen. Both of these spectra were dominated by contributions from the amino acids, tyrosine and phenylalanine, and the insoluble crystal spermine phosphate hexahydrate. The spectra of semen and seminal fluid were also very different from the spectra of sperm. It was determined that the contribution of seminal fluid dominates the Raman spectra of semen. This was further confirmed by analyzing Raman spectra of semen obtained from a donor who had had a vasectomy (azoospermia). These spectra very closely resembled those of semen and seminal fluid. The individual spectra from seminal fluid and azoospermic semen were correctly identified with a previously made chemometric model as semen. It was concluded that the presence of sperm is not necessary for the correct identification of semen using Raman spectroscopy and chemometrics. This further demonstrates a great potential of Raman spectroscopy as a universal tool for confirmatory identification of all main body fluids for forensic purposes.

*This project was supported by an award from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.*

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#### Semen, Raman Spectroscopy, Azoospermia



### E82 Phenotype Profiling Based on Raman Spectroscopy of Biological Stains: A Blood Test for the Donor Age

Igor K. Lednev, PhD\*, State University of New York at Albany, Albany, NY 12222; Kyle C. Doty, PhD, Silver Spring, MD 20904

**Learning Overview:** After attending this presentation, attendees will better understand the potential forensic application of Raman spectroscopy. The implementation of advanced statistics for the analysis of spectroscopic data and the evaluation of the accuracy and reliability of the conclusions made will be discussed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the accuracy and effectiveness of biological stain analysis for forensic purposes.

This presentation will report on the development of a universal, non-destructive, and confirmatory method for characterizing biological stains. The all-in-one method has a capability to identify the body fluid, determine human or animal origin, time since deposition and phenotype profile, race, sex, and chronological age, specifically.

Traces of body fluids discovered at a crime scene are a potential source of DNA, which is a major individual evidence in the modern forensic investigation. The application of Raman spectroscopy for non-destructive, confirmatory identification of biological stains at a crime scene, including dry traces of sweat, vaginal fluid, semen, saliva, and blood, has recently been reported.<sup>1</sup> The method allowed for differentiating animal and human blood as well as menstrual and peripheral blood.<sup>2,3</sup> In addition, the method was further developed for determining the time since deposition for bloodstains for up to two years.<sup>3</sup>

It would be of great help for criminal investigation to develop a phenotype profile immediately at a crime scene based on a rapid analysis of biological stains. With this goal in mind, the possibility of race and sex differentiation based on Raman spectroscopy of body fluid traces has been investigated.<sup>3</sup> Specifically, advanced statistical analysis of spectroscopic data was used to discriminate between Caucasian and African American donors based on dry peripheral blood traces. In addition, the differentiation of the donor's sex based on bloodstains and saliva traces, as well as race differentiation based on traces of semen, has been demonstrated.

The theory behind Raman spectroscopy is based on the inelastic scattering of low-intensity, non-destructive laser light by a solid, liquid, or gas sample. Very little or no sample preparation is needed, and the required amount of material tested with a Raman microscope can be as low as several picograms or femtoliters. A typical Raman spectrum consists of several narrow bands and provides a unique vibrational signature of the material. Typically, non-resonance Raman spectroscopy is not destructive for the sample. A portable Raman spectrometer is a reality now that should allow the identification at the crime scene.

In this proof-of-concept study, Raman spectroscopy and chemometrics have been used to analyze blood from human donors and differentiate between samples based on Chronological Age (CA).<sup>4</sup> Three groups of donors were selected including newborns (CA of <1 year), adolescents (CA of 11–13 years), and adults (CA of 43–68 years). A Support Vector Machines Discriminant Analysis (SVM) model was constructed, which demonstrated high accuracy in correctly predicting blood donors' age groups by showing the cross-validated sensitivity and specificity over 0.96. Overall, this preliminary study demonstrates the high selectivity of Raman spectroscopy for differentiating between blood donors based on the CA. The demonstrated capability completes this suite of phenotype profiling methodologies, including the determination of sex and race. CA determination has particular importance since this characteristic cannot be determined through DNA profiling, unlike sex and race. When completed, the developed methodology should allow for phenotype profiling based on dry traces of body fluids immediately at the scene of crime. The availability of this information within the first few hours since the crime discovery could be invaluable for the investigation.

*This project was supported by an award from the National Institute of Justice, Office of Justice Programs, United States Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.*

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#### Serology, Bloodstain, Phenotype Profile



## E83 Discovery Index Metabolites for the Estimation Age of Bloodstain

*You-Rim Lee, BS\**, Gyeonggi-do, Korea, SOUTH KOREA; *Ae Eun Seok, BS*, Eulji University, Gyeonggi-do 13135, SOUTH KOREA; *Jiyeong Lee, PhD*, Eulji University, Seongnam-si 13135, SOUTH KOREA; *Yoo-Jin Lee, MT*, Eulji University, Gyeonggi-do 13135, SOUTH KOREA; *Hyo-Jin Kim, MT*, Eulji University, Gyeonggi-do 13135, SOUTH KOREA; *Sora Mun, MT*, Eulji University, Gyeonggi-do 13135, SOUTH KOREA; *Hee-Gyoo Kang, PhD*, Eulji University, Gyeonggi-do 13135, SOUTH KOREA

**Learning Overview:** After attending this presentation, attendees will understand that bloodstain metabolites can be used to reliably index material for an estimation of a bloodstain age. Attendees will also learn that the age of a bloodstain can be distinguished weekly using candidate metabolites.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting candidate metabolites for estimating bloodstain age. This study is the first report to identify index materials used to analyze the age of bloodstains through metabolomics analysis.

In this study, index material for determining the age of bloodstains was discovered by analyzing changed patterns of bloodstain metabolites using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS). Bloodstains were prepared on filter paper and confirmed at weekly intervals until day 21. Using Venn diagrams and multivariate analysis, 62 candidate molecular features were selected.

Partial Least Squares Discriminant Analysis (PLSDA) was used to confirm that the group could be classified with an accuracy of 75.0%, and R2 and Q2 were 0.7513 and 0.6998, respectively. Among the 62 candidates, five metabolites were successfully identified. Metabolite A decreased as time passed. In the cases of metabolite B, metabolite C, and metabolite D, they increased at day 7 after day 0, and gradually decreased after day 7. In the simple regression analysis, all five candidates were statistically significant at day 21 versus day 0, and metabolite B and metabolite C at day 21 versus day 7 were also significant. The *p*-value of metabolite A was significant in all date combinations, so it is possible to distinguish time by week. Metabolite B and metabolite C were also statistically significant in all combinations, except day 14 versus day 7. Metabolite D showed significant results in all combinations, except day 7 compared to day 0, and metabolite D was significant at day 0 versus day 21, day 7 versus day 21, and day 14 versus day 21. Therefore, it is possible to measure the age of the bloodstain according to the distinguishing characteristics of candidate by date.

These novel metabolic approaches will help in the estimation of the age of bloodstains in forensic analysis. Currently, the time of the incident is specified through the estimated time of death by autopsy and forensic entomology. However, a metabolic approach can be used as the main basis for screening suspects by estimating the time of occurrence of the crime through the measurement of the age of bloodstains using the metabolites.

### Bloodstain, Bloodstain Age, Metabolomics



### E84 Veterinary Forensics: Animal Death Investigations and Veterinary Diagnostic Laboratory Personnel

*Megan Romano, DVM\*, University of Kentucky Veterinary Diagnostic Labor, Lexington, KY 40511; Michael S. Filigenzi, PhD, California Animal Health and Food Safety Laboratory, Davis, CA 95616; Robert H. Poppenga, DVM, PhD, California Animal Health and Food Safety Laboratory, Davis, CA 95616; Cynthia L. Gaskill, DVM, PhD, University of Kentucky Veterinary Diagnostic Labor, Lexington, KY 40511*

**Learning Overview:** After attending this presentation, attendees will understand the need for early communication and cooperation between law enforcement officials and veterinary diagnostic laboratory personnel in criminal animal death investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the important role of the veterinary diagnostic laboratory in a criminal animal death investigation. A 2018 case involving the death of a police dog will be presented.

In the spring of 2018, a 2-year-old intact male German Shepherd police dog was inside a 20'x20'x8' run in the center of a fenced yard while the dog's handler was away for several hours. The handler returned home and found the dog deceased. Several piles of vomit containing hot dog pieces were on the kennel floor. The handler had not given the dog any hot dogs in the past 48 hours. The handler rushed the dog to a veterinarian, who pronounced the dog dead. Malicious poisoning was strongly suspected due to the sudden death of a young, healthy dog, and the vomited hot dog pieces. The scene was photographed, and evidence was collected and taken to the state crime laboratory.

The veterinarian performed a gross postmortem examination. Tissue samples were collected, and formalin fixed. Fresh liver, heart blood, and urine were also collected. Samples and the dog's remains were submitted to a veterinary diagnostic laboratory for histological examination and toxicological analysis. The investigating detective requested analysis for drugs of abuse. The laboratory did not have the instrumentation to perform all requested analyses. The University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) Toxicology section was consulted and received samples from the initial veterinary diagnostic laboratory as well as the state crime laboratory. Urine and hot dog pieces were analyzed via Gas Chromatography/Mass Spectrometry (GC/MS), Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS), and high-resolution LC/MS. Very high concentrations of caffeine were detected in both the urine and the hot dog pieces. Lower concentrations of theobromine and theophylline were also detected in the hot dog. No illicit or prescription drugs, pesticides, herbicides, or other compounds detectable by these methods were present. Caffeine can cause agitation, tachycardia, vomiting, diarrhea, muscle tremors, and seizures in dogs. Caffeine can be lethal to dogs at high concentrations (minimum lethal dose in dogs is estimated at 110mg/kg body weight), and in this case was considered the likely cause of death. However, remains were not grossly examined by a board-certified veterinary pathologist, and histological examination was limited to samples collected by the veterinarian. Potentially valuable samples were stored inappropriately, misidentified, or not collected. Only one sample—the hot dog pieces from the state crime laboratory—arrived with a chain of custody. In animal cruelty cases, animals and excretions are important evidence. Following proper evidence handling protocol ensures the best chance of a successful prosecution.

Early consultation with veterinary diagnostic laboratory personnel in criminal animal death investigations is critical to ensure appropriate sample collection, storage, and analysis. A thorough postmortem examination by a qualified veterinary professional (ideally a board-certified veterinary pathologist) is vital to determining cause and manner of death. A complete, accurate history is essential to interpret postmortem examination findings and to direct additional testing. Communication and collaboration between police and veterinary diagnostic laboratory personnel from the outset ensures the best results in criminal animal death investigations.

#### **Veterinary Forensics, Animal Cruelty, Veterinary Diagnostic**





## E85 A Validation Study on Automated Groove Detection Methods in 3D Bullet Land Scans

Kiegan Rice, MS\*, Center for Statistics and Applications in Forensics, Iowa State University, Ames, IA 50011; Heike Hofmann, PhD, Center for Statistics and Applications in Forensic, Iowa State University, Ames, IA 50011; Ulrike Genschel, Center for Statistics and Applications in Forensics, Iowa State University, Ames, IA 50011

**Learning Overview:** After attending this presentation, attendees will understand the proposed pre-processing approach, as well as be aware of outcomes from automated comparison methods from a variety of validation tests on different bullet types.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a new groove detection and removal process which leads to higher accuracy than currently implemented methods and reduces error rates in the automated comparison process on a variety of bullet types.

The prevalence of research on the automated comparison of bullet marks has increased in the past several years, particularly following the 2016 President's Council of Advisors on Science and Technology (PCAST) report on the validity of feature-comparison methods in forensic science. The main avenue being pursued in this research area is the use of statistical models applied to high-resolution 3D scans of Land Engraved Areas (LEAs). An important step in introducing these new automated methods is ensuring accurate data pre-processing techniques.

Being able to correctly identify between data from LEAs and Groove Engraved Areas (GEAs) turns out to be the single, most important step in data pre-processing. Dealing with these areas separately is crucial to ensure good model fits in subsequent processing steps. Removal of data from GEAs significantly reduces the amount of noise in the characteristics used in automated comparisons. Failure to correctly identify and remove data from GEAs can lead to misidentification of these characteristics.

Distinguishing between LEAs and GEAs is a problem at which human vision excels, but it is quite challenging for automatic procedures due to the nature of the data collected: the bullet curvature presents the main structure in the data, but the abrupt change between LEAs and GEAs introduces a competing structure. This overwhelms standard modeling techniques. This study employed pre-processing techniques based on robust statistical methods to distinguish between LEAs and GEAs. Techniques from robust statistical methods allow the algorithm to focus on the main structure and separate out elements from the secondary structure of the GEA.

Results from these techniques showed early promise when applied to the closed-set study of the Hamby Set 44 bullets.<sup>1</sup> The pre-processing methods are evaluated at different levels. In the first step, predicted results from the techniques were compared to manually identified groove locations. The second, and perhaps more important step, was to assess the performance of a method in regard to the prediction accuracy of the automated matching algorithm as described in Hare, Hofmann, and Carriquiry.<sup>2</sup>

This study is using closed-set and open-set studies to evaluate the effectiveness and accuracy of the proposed techniques. Results are based on publicly available data from the National Institute of Standards and Technology (NIST) Ballistics Research Database as well as studies from collaborating forensic laboratories and departments across the United States. This additional validation step tests the proposed method's ability to transfer to different types of bullets and rifling methods and highlights limitations.

This litany of validation steps ensures a safe use of the proposed technique in fully automating the process and removing the need for human intervention in the data pre-processing.

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### 3D, Firearms, Validation

### E86 Discrimination Between Aerial and Targeted Shooting by the Analysis of Trace Evidence: A Case Study

*Khalid S. Feras, MPhil\**, Punjab Forensic Science Agency, Lahore, Punjab 54000, PAKISTAN; *Mohammad A. Tahir, PhD\**, Punjab Forensic Science Agency, Lahore, Punjab 54500, PAKISTAN; *Mudassar Baig, MSc\**, Punjab Forensic Science Agency, Lahore, Punjab 54000, PAKISTAN; *Abdul Razaq, MPhil\**, Punjab Forensic Science Agency, Lahore, Punjab 54000, PAKISTAN; *Muhammad Irfan Ashiq, PhD\**, Punjab Forensic Science Agency, Lahore, Punjab 54500, PAKISTAN; *Zumrad Usman Bhutta, MS\**, Sargodha, Punjab 40100, PAKISTAN

**Learning Overview:** After attending this presentation, attendees will understand the use of trace evidence analysis to establish trajectory of the bullet and to discriminate between aerial and targeted shooting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the use of Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDX) for the analysis of trace evidence trailing on the bullet and to reach a definite conclusion regarding aerial or targeted shooting.

A suspected shooting took place in the area around the residence of a famous personality from the Pakistani judiciary. The matter was highlighted in the media and led to chaos. It was suspected to be a targeted shooting. However, no loss of life or injury to the residents took place. The matter was taken up by the provincial government and the crime lab was requested to unveil the facts behind the incident. The task was to determine that either the incident was some terrorism activity (targeted firing) or the bullets could have been the result of some aerial shooting (i.e., stray bullets).

The crime scene investigation team found two bullets, one (30-bore pistol) bullet found in the garage and the second (9mm pistol) found in the backyard area shaded by a fiberglass shed. The team noticed a suspect hole in the fiberglass just above the area where the 9mm bullet had landed. Both bullets and a portion of the fiberglass that had the suspected piercing damage in it were submitted to the trace chemistry department to determine whether these were the result of targeted shooting or were stray bullets.

Keeping in mind the case scenario, an analysis strategy was devised to establish the trajectory/path that both the bullets could have followed to approach the target. The bullets were initially examined through low power magnification light microscopy followed by SEM/EDX to observe the surface of the bullets for the severity of impact and for the detection and identification of any traces of building material, vegetation, paint, or something else adhering to the bullets. No significant damage (deformation) was observed on either bullet tip, which could have come about as the result of targeted firing impacting the hard targets. However, the SEM/EDX examination revealed that the 9mm pistol bullet had some greenish material impinged into the damage site of the bullet, which was identified as the fiberglass through SEM/EDX. A relatively minor bullet defect is an indication of possible aerial shooting because the velocity of the bullet may have been slowed due to air drag, and the speed was further reduced by striking and passing through the fiberglass shed. Whereas, the second 30-bore pistol bullet was found to have only traces of silicate (i.e., soil material) adhering to the side of the tip along a soft depression due to the free-fall parabolic path impact on the ground.

The SEM/EDX analysis of aluminum stubs coated with pure carbon adhesive prepared by a single perpendicular dab just on and around the defect hole in the fiberglass revealed the presence of copper and consistent Gunshot Residue (GSR) particles. The presence of GSR particles around the hole in the fiberglass sheet of the shed indicates that the hole had been sourced from the bullet during its entry through the fiberglass sheet.

The bullet-intermediate target interaction, path of the travelled bullets, origins of trace evidentially valued deposits on bullet, traces of the bullet jackets and the gunshot residues due to the bullet wipe on the fiberglass shed, and, finally, the extent of bullet's surface deformation demonstrates that the incident was an aerial shooting as opposed to a targeted shooting.

#### Forensic Investigation, Trace Evidence, Aerial/Targeted Shooting



### E87 The Forensically Relevant Applications of Laser Ablation Direct Analysis in Real Time Imaging-Mass Spectrometry (LADI-MS)

Kristen L. Fowble, BS\*, State University of New York at Albany, Albany, NY 12222; Rabi A. Musah, PhD, State University of New York at Albany, Albany, NY 12222

**Learning Overview:** The goals of this presentation are to introduce attendees to: (1) the basics of the new imaging mass spectrometry technique known as LADI-MS; (2) its wide applicability to small molecule spatial distribution mapping in forensic sample types, including psychoactive compounds in latent fingerprints and inks on paper; and (3) its non-imaging capabilities, including derivatization of the psychoactive component of psilocybin mushrooms (i.e., psilocybin).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a new technique that is being developed for potential integration into crime laboratories and research institutions. The wide applicability of the technique means that a single instrument could tackle a number of forensic sample analyses, thus reducing the cost and training that would be required for multiple instruments.

Imaging mass spectrometry provides a means to obtain chemical composition information while retaining the spatial distribution of the detected analytes in the sample of interest, thus providing two types of information that would typically require multiple instruments. Most current imaging techniques require the use of solvent, high vacuum, and/or the application of a matrix. However, these steps can complicate the ability to routinely detect and map the spatial distribution of small molecules and can also be time-consuming and contribute to casework backlogs. It is demonstrated that using an approach that integrates a Direct Analysis In Real Time (DART<sup>®</sup>) ionization source, an ultraviolet neodymium-doped yttrium aluminum garnet (Nd:YAG) laser ablation system and a Time-Of-Flight (TOF) mass spectrometer, small-molecule spatial distribution maps can be acquired for a broad range of sample types, with no sample pretreatment requirements.

The ability of the technique to detect small molecules within latent fingerprints was demonstrated using prints that were deposited after exposure of hands to psychoactive plant material (*Piper methysticum*), cocaine, or pseudoephedrine. LADI-MS revealed spatial distributions of endogenous cholesterol, localized to fingerprint ridges, while providing confirmation of exposure to the psychoactive small-molecule cocaine, kavain (derived from *P. methysticum*), and pseudoephedrine. The spatial distribution mapping of cholesterol in a lifted print on the non-conductive adhesive side of tape was also accomplished. The ion images obtained of the endogenous compounds revealed the fingerprint ridge pattern necessary for identification of an individual.

The approach was also applied to the detection of differently sourced inks on paper. A peace sign image was drawn using two different brands of black markers (one used to draw the outer circle and the other, the inner lines). LADI-MS small-molecule spatial distribution maps allowed the two inks to be distinguished based on the chemical composition. This technique was also applied to numbers that were subsequently altered with a second pen to form a different number (i.e., number 1 changed to 7). This technique would be useful in determining alterations made to a document using differently formulated inks.

The robust LADI-MS system can also be utilized for non-imaging experiments. Typically, the psychoactive compound psilocybin undergoes dephosphorylation to psilocin during traditional chromatographic-mass spectrometric analysis. To confirm its presence in a sample, derivatization is required, thus prohibiting the ability to detect the intact molecule in imaging experiments of psychoactive mushrooms. Here, the laser was used for the instantaneous derivatization of psilocybin directly on the sample by firing at a drop of psilocybin solution and the derivatizing agent (*N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide) on the surface of a piece of cloth. Both psilocybin+2TMS and psilocybin+3TMS were detected by LADI-MS. This could lead to the potential imaging of psilocybin spatial distributions in intact mushroom material by spraying the derivatizing agent on the surface of mushroom samples prior to imaging experiments.

The newly developed LADI-MS technique can be applied to a number of forensic sample types, including latent fingerprints, inks on paper, and derivatization of psilocybin. It is shown to be a powerful tool for the detection and mapping of a range of small molecules in a variety of sample types with no sample preparation steps.

#### Imaging Mass Spectrometry, Latent Fingerprints, Inks on Paper



### E88 How Do Latent Print Examiners Perceive Proficiency Testing? An Analysis of Examiner Perceptions, Performance, and Print Quality

Sharon Kelley, PhD\*, Institute of Law, Psychiatry, and Public Policy, Charlottesville, VA 22903; Daniel C. Murrie, PhD, Institute of Law, Psychiatry, & Public Policy, Charlottesville, VA 22908; Brett O. Gardner, PhD, University of Virginia, Charlottesville, VA; Karen D.H. Pan, Department of Statistics Halsey Hall, Charlottesville, VA 22904; Karen Kafadar, PhD, University of Virginia, Charlottesville, VA 22904; Kellyn Blaisdell, BA, University of Virginia, Charlottesville, VA 22902

**Learning Overview:** The goal of this presentation is to educate attendees on how latent print examiners view current proficiency testing items and how such views relate to more objective measures of proficiency tests, such as print quality metrics and examiner test performance.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by shedding light on the current state of latent print proficiency testing as viewed by its primary consumers (i.e., latent print examiners). This presentation will investigate the connection between these views and the results of objective evaluative measures (i.e., print quality metrics). Both these views and the objective measures ultimately will inform efforts to craft more rigorous, or better operationalized, materials for training and assessing proficiency.

Accrediting agencies generally require that forensic science laboratories administer proficiency tests, or practice tests designed to resemble real forensic casework, at regular intervals to assess analysts' competence. Moreover, latent print examiners sometimes cite results on proficiency tests as evidence that the work in an actual case is accurate.<sup>1</sup>

Importantly though, commercial test materials may differ in important ways from "real world" evidence.<sup>2,3</sup> If proficiency tests are simpler than actual casework, the value may be diminished. On the most recent latent print examination proficiency test administered by Collaborative Testing Services, Inc. (CTS), 91% of respondents received a score of 100%.<sup>4</sup> This study may reveal important ways in which proficiency tests do not provide true metrics of competence or proficiency in the field. Without such metrics, both internal (e.g., by laboratory managers) and external oversight (e.g., by accrediting bodies) become less meaningful.

For the present study, CTS added a series of questions to its latent print examination proficiency test shipped in August 2017. Three hundred twenty-two latent print examiners submitted completed tests and answered attached survey questions during the testing period. The survey asked respondents to separately rate the level of challenge and similarity to casework of each latent print on the test using an 11-point scale ranging from 0=*Extremely easy/Nothing like casework*, to 10=*Extremely challenging/Exactly like casework*. Additionally, examiners identified the *least* and *most* challenging latent print and rated their confidence in the accuracy of their decisions. For the latent print that examiners identified as most challenging, examiners identified the characteristic(s) that caused the print to be challenging. Finally, to provide a more objective measure against which to evaluate the proficiency test and examiners' perceptions of the test, all prints used in the test were examined using a global-quality metric algorithm (i.e., LQM).<sup>5</sup>

In brief, the mean level of perceived difficulty across all items was 4.27, indicating that, in general, participants found the questions to be relatively easy. Examiners also typically perceived latent prints in the test to be similar to their casework ( $M=6.97$ ). Interestingly, items perceived as more similar to casework were also perceived as more difficult to complete ( $r[308]=0.30, p<.001$ ). Examiners expressed significant confidence in conclusions regarding both the least and most challenging items on the test.

In general, all included prints scored highly on a metric examining level of image quality. Indeed, no latent print scored below a 60 out of 100 and the estimated probabilities that an examiner would find each print of value for identification and comparison were 95% or greater for all prints included in the test. The quality metric scores for each latent print and its source print were averaged to compute an overall quality score for each test item. This quality score was not significantly correlated with examiners' perceptions of item difficulty ( $r[7]=-0.68, p=0.09$ ) but was significantly correlated with perceptions of item similarity to casework ( $r[7]=-0.79, p=0.04$ ). Additional analyses exploring examiner perceptions and related associations with objective print quality and test performance will be discussed.

In conclusion, the current findings reveal that examiners viewed proficiency test items as generally easier than casework, were highly confident in the conclusions, and achieved very high accuracy in the conclusions. Quality metric scores corroborated examiners' perceptions in that included prints were consistently rated as very high quality. Given that examiners viewed more challenging prints as more similar to typical casework, the results have important implications for meaningful proficiency testing moving forward.

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#### Latent Print Examination, Proficiency Testing, Quality Metrics



## E89 How Does Training and Certification Affect the Perception of Accuracy During Fingerprint Examinations?

Crystal L. Wagoner, MFS\*, Clarksville, TN 37043

**Learning Overview:** After attending this presentation, attendees will understand the different training and certification requirements of latent print examiners at local, state, federal, and private agencies, and how that training and/or certification impacts the individual fingerprint examiner's perception of accuracy during fingerprint examinations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the decision-making process for standardization of training and certification for latent print examiners and providing recommendations for future research in this area. This study could potentially be expanded to include the training and certification requirements for other fields in forensic science.

For more than 100 years, fingerprints have been used as a means of human identification.<sup>1</sup> Fingerprint technology is one of the oldest disciplines in forensic science and has been accepted in the court system for more than a century.<sup>1-4</sup> However, several high-profile cases have highlighted the potential for human error during the identification process, and the reliability of latent print identifications and other forensic sciences are now being challenged.<sup>5-8</sup> However, very little research exists to determine if differences in training or certification have any effect on the accuracy of fingerprint examinations.

A quantitative correlational study was conducted to examine the training and certification process for fingerprint examiners at various levels of law enforcement (local, state, federal, and private) and to determine what effect, if any, these differences had on the accuracy of conclusions drawn during fingerprint identifications. A total of 404 fingerprint examiners from the American Academy of Forensic Sciences (AAFS) or the International Association for Identification (IAI) participated in an online questionnaire. The data obtained from that questionnaire was analyzed using the latest SPSS software and a Pearson's Chi-square.<sup>9</sup>

The results of the study indicated a significant correlation between the level of agency and the type of training a fingerprint examiner was required to complete. There was also a significant correlation between the level of the agency and a requirement for certification. No significant correlation was found between differences in training and the accuracy of fingerprint examinations. However, logistic regression analysis suggested that fingerprint examiners who received Outside Training, and a combination of Outside Training with On the Job training were significant predictors of a perception of accuracy by fingerprint examiners. Finally, there was a significant correlation between the requirement for certification and the perception of accuracy during fingerprint examinations.<sup>9</sup>

Identifying the training and certification procedures that result in the greatest accuracy and reliability of fingerprint examinations will reinforce the credibility of fingerprint examinations as a means of identification and pave the way for recommendations of standardized training and certification. This presentation will demonstrate the inconsistencies that exist in the training and certification of fingerprint examiners and the areas that need more research.

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### Latent Print Examiners, Training, Certification



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### E90 What Do Latent Print Examiners Want in a Statistical Model?

*Anthony Koertner, MS\*, Forest Park, GA 30297*

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**Learning Overview:** After attending this presentation, attendees will have gained knowledge on the various statistical models currently in existence in the latent print community. This presentation will discuss scores derived from various statistical and probabilistic models and how they fit in the many available Bayesian Verbal Equivalent Scales utilized not only in the forensic sciences but other industries as well. Attendees will also be more familiar with recently published research surrounding how potential jurors interpret scores derived from a particular latent print statistical model.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing current progress and inherent limitations of proposed statistical approaches to quantify fingerprint evidence with the intent to elicit candid discussion on what can realistically be achieved at this time and whether these limitations are “true” issues or merely byproducts of any acceptable scientific method. Shedding light on these perceived limitations may assist latent print examiners in their understanding of these statistical models and the imperfections and may be somewhat suitable in an attempt to satisfy some of the demands set forth by the National Research Council (NRC) and the President’s Council of Advisors on Science and Technology (PCAST).

The search for a suitable latent print statistical model to assist in expressing the weight of friction ridge evidence has intensified since the 2009 NRC Report on Forensic Science, *Strengthening Forensic Science in the United States: A Path Forward*. The NRC report, along with the 2016 PCAST report, *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*, challenged the friction ridge community to become more objective and develop tools to express the strength of evidence that is communicated to stakeholder communities. As practitioners, there is a tendency to be concerned with how evidence can best be presented in a trial format in a manner that accurately conveys the strength of the evidence and is understandable by a jury.

From a practitioner perspective, there should be a model that: (1) supports an expert’s opinion of source attribution, (2) does not over- or understate the strength of the evidence, (3) shields from *any* potential error, (4) provides the exact same results every time, and (5) is entirely objective. While all these criteria may be desired, can or will these requirements be met? Barring these achievements, are any statistical models “useless” or do practitioners need to curb expectations of what can be realistically achieved within the acceptable limits of science?

*The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the United States Department of Defense or United States Department of the Army.*

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#### **Fingerprint, Statistics, Model**



### E91 Plato: Unaware Narrator of a Death by Hemlock Poisoning—From Socrates' Death to the Present

*Isabella Aquila, MD\**, Institute of Legal Medicine, Catanzaro 88100, ITALY; *Matteo A. Sacco, MD\**, Chair of Legal Medicine, University of Catanzaro, Catanzaro 88100, ITALY; *Roberto Raffaele, BE\**, University Magna Graecia of Catanzaro, Crotona, ITALY; *Santo Gratteri, MD*, Viale Europa, Germaneto, Catanzaro 88100, ITALY; *Pietrantonio Ricci, MD, PhD*, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY

**Learning Overview:** After attending this presentation, attendees will better understand poisoning by hemlock with the analysis of an emblematic historic case—the death of Socrates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by underlining the uniqueness of the Platonic writing in the tale of a rare case of poisoning by hemlock.

Poisonings are classified according to the manner in which they occur. The forensic sciences deal with the deaths that occur with violent modality, such as suicides or homicides. Poisoning by hemlock has special toxicological characteristics. Hemlock (*Conium maculatum* L. *Umbelliferae*) has long been known as a poisonous plant, belonging to the Apiaceae family.<sup>1</sup> The toxicity of hemlock is due to the 5-piperidine-type coniine alkaloids, including coniine and  $\gamma$ -coniceine. The dried plant contains the highest concentration of coniine, while the fresh plant material tends to contain the most  $\gamma$ -coniceine. The action of coniine alkaloids is intended to stimulate and then paralyze the nicotinic receptors. The  $\gamma$ -coniceine is among the most toxic alkaloids biosynthetically formed. The poisoning in humans can be caused by ingestion of the plant. No forensic cases of poisoning by hemlock have been reported. For this reason, this study reports on a historic and emblematic case of this type of poisoning through the historical-scientific analysis of the death of Socrates.

A review of literature was performed on the search engine PubMed NCBI inserting the keywords “Socrates” and “Hemlock & *Conium maculatum* death.” Socrates (Athens, 469-399 BC), son of a sculptor and a midwife, dedicated himself from an early age to philosophy. After the fall of the Thirties, in 399 BC, he was subjected to a political process with the charge of impiety (not believing in the gods of the state) and moral corruption of the young disciples. It is probable that the accusers intended only to exile him, but Socrates refused the compromises and renounced the opportunity of escape. Plato, in *Phaedo*, describes in detail, through the eyes of Critone, the death of Socrates. In the conclusive lines, it reads that a servant entered the room with the prepared drug, pounded into a cup. Socrates, after drinking it, began to feel heavy legged and began to lie on his back. Meanwhile, the Executioner was examining his feet and legs through the application of painful stimuli in order to see if the pain was perceived. By now, cold had come to his belly and, a little later, the widened eyes of Socrates remained open and fixed. This description, quite detailed even considering the era in which it was written, currently remains the only report in the literature.

It would appear that the poisoning of Socrates represents to date, after more than 2,000 years, the first case with forensic aspects regarding the manner of death in history, primarily because it was a capital punishment. The scientific articles published to date have focused in particular on chronic hemlock poisoning in cattle, on chemical characteristics of the alkaloids, or on cases of accidental poisonings. General symptoms of poisoning by hemlock are represented by effects on the central and peripheral nervous system and the autonomic nervous system ganglia with vomiting, tremor, rhabdomyolysis, ataxia, headache, acute renal failure, rapid breathing, ipsalivation, nausea, convulsions, and coma until the fatal outcome. The alkaloids are volatile and produce a characteristically unpleasant odor that can be observed in the breath and urine of poisoned individuals. The progressive centripetal paralysis is characteristic of poisoning by hemlock.<sup>2,3</sup> This data makes it doubtful hemlock was used in the death of Socrates, according to some authors, especially noting the absence of the description of unpleasant odor and the common gastrointestinal effects of the poison. However, it is believed that Plato gave a modified side of the story about the death of Socrates for political, apologetic, and philosophical reasons, describing a more “noble” death. Through looking at the past, the case of Socrates is unique for its characteristics, but, above all, because it represents to date the only example of poisoning by hemlock of historical and forensic interest present in ancient literature.

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#### Forensic Science, Socrates' Death, Poisoning



**E92 WITHDRAWN**





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### E93 The Classification of an Ethanol Coeluting Compound Via Headspace Gas Chromatography (HS/GC)

*Andrea Kardohely\**, Canton, OH 44718; *Jay D. Spencer, MBA*, Canton-Stark County Crime Laboratory, Canton, OH 44707; *Jennifer Creed, BS*, Canton-Stark County Crime Laboratory, Canton, OH 44707; *Michele M. Foster, BS*, Canton-Stark County Crime Laboratory, Canton, OH 44707; *Lauren L. Richards-Waugh, PhD*, Marshall University Forensic Science Program, Huntington, WV 25701

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**Learning Overview:** After attending this presentation, attendees will understand how common inhaled anesthetics for general hospital anesthesia interact with routinely encountered volatile compounds.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the issues that can arise with the presence of an inhaled anesthetic when analyzing a sample for ethanol concentration under HS/GC.

Blood alcohol analysis plays an important part in the legal realm. Alcohol analysis in a crime laboratory is performed to determine the concentration of ethanol present in a biological sample for criminal and death investigations. In driving under the influence cases, the ethanol concentrations are the basis for filing criminal charges. In a blood sample tested by the Canton-Stark County Crime Laboratory (CSCCL) for criminal investigation, an unknown compound coeluted with ethanol under HS/GC.

Based on the information provided by the investigating officer, the individual from whom the sample was collected was being prepared for surgery while the sample was drawn; therefore, the unknown compound was thought to be an inhaled anesthetic. Inhaled anesthetics administered during preparation for surgery are very volatile. Desflurane, isoflurane, and sevoflurane are commonly available inhaled anesthetics. These anesthetics were analyzed following the CSCCL ethanol analysis protocol used to determine Blood Alcohol Content (BAC), and it was discovered that sevoflurane had an almost identical retention time as ethanol. Sevoflurane was observed as a shoulder on the ethanol peak using the BAC1 column. Sevoflurane and ethanol completely coeluted on the BAC2 column, which was comparable to the casework sample analyzed by the CSCCL.

In addition, the three anesthetics were evaluated against other common volatile substances (methanol, isopropanol, and acetone) included with ethanol in the laboratory's volatile mixture standard. While sevoflurane coeluted with ethanol, the other two inhaled anesthetics coeluted with two other volatile compounds. Desflurane partially coeluted with methanol on the BAC1 column, but separation was achieved with the BAC2 column. Isoflurane appeared as a shoulder on the isopropanol peak, partially coeluting on both BAC1 and BAC2 columns.

By decreasing the temperature of the GC oven from 40°C to 26°C, separation was achieved between the sevoflurane and ethanol peaks. At this time, the method for the separation of the compounds was not able to be validated due to problems with the bias for the ethanol quantitation results. Future research would include improving the bias, performing a full-method validation, and separating the two compounds on different columns and instrumentation in order to observe any variation.

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#### Ethanol Analysis, Inhaled Anesthetics, Coelution



### E94 Illicit Drug Deaths in Older Adults: The Need for Comprehensive Scene Investigation

*Stanislaw T.J. Bielous, BS\**, Syracuse, NY 13210; *Dennis J. Chute, MD*, Dutchess County Medical Examiner's Office, Poughkeepsie, NY 12601; *Veronica Salvas, MPH*, Dutchess County Medical Examiner's Office, Poughkeepsie, NY 12601; *Robert J. Bready, MS*, Dutchess County Medical Examiner's Office, Poughkeepsie, NY 12601; *Kia K. Newman, MD\**, Dutchess County Medical Examiner's Office, Poughkeepsie, NY 12601

**Learning Overview:** After attending this presentation, attendees will: (1) have an increased awareness of the often-overlooked area of older adult illicit drug use, (2) understand the importance of approaching every death scene with a reasonable amount of suspicion, and (3) understand the need for comprehensive scene investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by shining a light on the growing trend of illicit drug use in older adults and will present case studies demonstrating how a thorough scene investigation can prevent the misclassification of substance abuse-related deaths.

Drug use in older adults is a growing issue within both the legal and medical communities. Studies conducted in the early 2000s predicted that the number of adults over 50 years of age with substance abuse issues would almost triple from 1.7 million in 1995 to an estimated 4.4 million in 2020.<sup>1,4</sup> This dramatic rise is attributed to both a 50% increase in the over-50 population and a 70% increase in the rate of substance abuse.<sup>1</sup> It is thought that due to pharmacokinetic changes associated with age, older individuals are more sensitive to adverse side effects of drug use, such as reduced physical functions and an increased risks of falls.<sup>2,3</sup> In a review of all substance abuse-related deaths in Dutchess County, NY, between 2010 and 2017 ( $n=436$ ), it was found that adults over 50 years of age accounted for 29% of all accidental drug overdose deaths; the oldest illicit drug case was 75 years old.

Death scene investigations are an integral step in establishing the circumstances and causes of sudden and unexpected deaths investigated by medical examiner and coroner offices. Many times, death of individuals over 50 years old appear natural due to concurrent health conditions. However, it is critical that each scene be approached with an appropriate level of suspicion. In such cases, the possibility of illicit drug abuse may seem unlikely at first, and paraphernalia may have been removed or hidden from view by family or friends. Presented here are three cases that may have been released at the scene and attributed to natural causes. However, a comprehensive scene investigation revealed evidence of drug use and an autopsy was performed.

**Case 1:** A 67-year-old female was found naked on the toilet. She had a history of chronic obstructive pulmonary disease and recent complaints of body aches. Without proper scene investigation, this decedent's cause of death may have been erroneously attributed to her natural disease. A meticulous scene investigation revealed white powder in a covered, decorative bowl, marijuana, juice box straws with white residue, and other paraphernalia. An autopsy showed pulmonary emphysema, bronchiolitis, and atherosclerotic cardiovascular disease. However, toxicology revealed THC, cocaine, and codeine in her blood; her death was certified as due to acute cocaine intoxication.

**Case 2:** A 66-year-old male was found on his knees in his apartment. A careful review of the decedent's medications revealed a white plastic straw, white residue, and several glassine envelopes hidden within a prescription bottle. Autopsy revealed hypertensive and atherosclerotic cardiovascular disease and obesity. Toxicology results showed numerous substances and his death was certified due to acute fentanyl, heroin, and alprazolam intoxication.

**Case 3:** A 61-year-old male with a history of diabetes and mental health issues was found dead, lying supine on the floor of his bedroom. A careful investigation of the scene revealed a white powder that appeared to be either crushed medication or cocaine and signs of oxycodone abuse. An autopsy showed coronary artery atherosclerosis. However, toxicology revealed alprazolam, cocaine, morphine, oxycodone, and fentanyl, and his death was certified due to acute opioid, alprazolam, and cocaine intoxication.

Illicit drug use in older adults is an issue that will continue to grow and subsequently be reflected in the caseloads seen by medical examiners. These case studies highlight examples of how comprehensive scene investigation helped identify illicit drug use in atypical scenarios and thus averted the incorrect assumption of an older adult death as being natural. These circumstances present just one reason well-trained medicolegal death investigators are needed to perform comprehensive scene investigations. It is vital that investigators understand the growing prevalence of drug use in older adults and become proficient in recognizing the signs of drug use within this population.

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#### Illicit Drug Deaths, Older Adults, Death Scene Investigation



### E95 I Smell a Rat: Optimizing a Method for Detecting the Rat Poison Brodifacoum With Gas Chromatography

Abigail L. Meyer, BS\*, Medina, OH 44256; Morgan M. Clothier, MFS, DC Department of Forensic Sciences, Washington, DC 20024; Brandon P. Jones, MS, DC Department of Forensic Sciences, Washington, DC 20024; Luke C. Short, PhD, DC Department of Forensic Sciences, Washington, DC; Lauren L. Richards-Waugh, PhD, Marshall University Forensic Science Program, Huntington, WV 25701

**Learning Overview:** After attending this presentation, attendees will understand how to detect brodifacoum, a type of rat poison that has recently gained notoriety as an emergent adulterant to illicitly purchased controlled substances, using Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography with Flame Ionization Detection (GC/FID). It is imperative that both GC/MS and GC/FID, two gold standards within the field of forensic chemistry, are capable of detecting this lethal compound. A method for detecting brodifacoum will be discussed by varying several instrument parameters (e.g., column length and inlet temperature). As proof of principle, the optimized method will then be applied to mock case samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a validated method to target this emergent adulterant. Forensic methods often trade run time by sacrificing detection of these later-eluting compounds. Such rapid schemes run at odds of assessing the potential presence of brodifacoum. Further, there is a paucity of modern literature to detail the analytical detection of this compound, or even related compounds, via GC. Thus, available methods discussed in literature are not germane to current analytical instrumentation.

Brodifacoum is currently a common rodenticide belonging to the class of pesticides known as “superwarfarins.” These compounds were developed from toxicants of warfarin, a response to the increase in warfarin-resistant rats and mice. Brodifacoum is a coumarin anticoagulant, causing hemorrhaging and severe vitamin K deficiency after prolonged exposure. It has the potential to be lethal both to rodents and to humans due to its long half-life and, thus, residency within the body.<sup>1</sup>

In early 2018, Illinois health care providers reported multiple cases of patients with unexplained bleeding who had also self-reported as using illicit substances, specifically synthetic cannabinoids. Further clinical testing detected the presence of brodifacoum as a likely causative factor in hemorrhaging, which was later found to be present in tested synthetic cannabinoid samples.<sup>2</sup> Over the following months, more than 200 clinical cases of brodifacoum poisoning resulting from synthetic cannabinoid use were reported in nine states.<sup>3</sup> While exposure to brodifacoum is only seldom lethal, the treatment is prolonged and requires hospitalization.<sup>4</sup>

In Washington, DC, the use of synthetic cannabinoids has significantly escalated since 2015. For example, over a two-week peak period in July 2018, DC Fire and Emergency Medical Services (EMS) reported more than 450 calls for suspected synthetic cannabinoid overdoses, including some resulting in fatalities.<sup>5</sup> It is imperative that forensic science laboratories in the region and other cities across the country are equipped with optimal methods for detecting brodifacoum. Such detection techniques will better equip local public health officials with the tools to detect this toxic compound, as well as other poisons, as these emerge in the local illicit controlled substances.

This research is directed at solving not just a validation scheme, but also to provide the forensic chemist a method to be used on real-world samples. Initial analyses were performed using a sample of brodifacoum in various solvents. Different methods and different column lengths were tested using both GC/MS and GC/FID. Additionally, the inlet temperature was adjusted to account for any possible thermal decomposition of brodifacoum. Once optimal parameters were established, non-controlled plant material was adulterated with brodifacoum, synthetic cannabinoids, and various combinations of both in order to mimic real-world scenarios. Further analyses will seek to establish the optimal method for detecting brodifacoum and other similar rodenticides in combination with common spices and controlled substances.

The initial screen for brodifacoum in acetone using GC/MS produced two late eluting peaks; these results were consistent for all methods and column lengths tested. The results were also repeatable when analyses were conducted in a variety of solvents. An observed decrease in peak area indicated that inlet temperatures greater than 260°C resulted in the breakdown of brodifacoum. When analyzed with GC/FID, two late eluting peaks were also present. The results of both GC/MS and GC/FID will be compared to determine which approach is optimal for detecting trace amounts of brodifacoum, but additionally, both instruments will be demonstrated to function to successfully presumptively detect and confirm the presence of brodifacoum in samples, a requirement for confirmation under the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) guidelines.

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1. Douglas Feinstein, Sergey Brodsky, Guy Weinberg, Richard van Breeman, and Israel Rubinstein. Brodifacoum Poisoning: A Clear and Present Danger to Public Health in the USA. *Toxicology Letters*, no. 268 (2017): 71-72.
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5. Marisa Peñaloza. D.C. Has Had More Than 300 Synthetic Marijuana Overdoses in 2 Weeks. *National Public Radio*, last modified July 27, 2018.

#### Brodifacoum, Synthetic Cannabinoids, Gas Chromatography

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## E96 Method Validation for the Detection of 22 Benzodiazepines, Including Clonazolam, Etizolam, and Flubromazolam, Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Rachel Lane\*, Huntington, WV 25701; Dustin Smith, BS, South Carolina Law Enforcement Division, Columbia, SC 29212; Jared Castellani, MS, South Carolina Law Enforcement Division, Columbia, SC 29212; Lauren L. Richards-Waugh, PhD, Marshall University Forensic Science Program, Huntington, WV 25701

**Learning Overview:** After attending this presentation, attendees will understand the analytical method for the determination of benzodiazepines, including clonazolam, etizolam, and flubromazolam, in human whole blood.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a reliable method for quantifying the target compounds by incorporating the analysis of emerging designer benzodiazepines into a previously accepted method for the analysis of classic benzodiazepines and their metabolites.

Benzodiazepines are a heterocyclic class of drugs used to treat a range of conditions, including anxiety, insomnia, and alcohol withdrawal.<sup>1</sup> In the United States between 1996 and 2013, the number of adults who filled a benzodiazepine prescription increased from 8.1 million to 13.5 million.<sup>2</sup> Benzodiazepine dosage also increased by more than triple during this period.<sup>2</sup> From 2002 to 2015, there was a 4.3-fold increase in the total number of deaths involving benzodiazepines.<sup>2</sup> The adverse effects of benzodiazepine abuse and addiction continue to be overlooked, most likely because 75% of deaths involving benzodiazepines also involve an opioid.<sup>2</sup> Designer benzodiazepines are structural analogs of controlled benzodiazepines that have been synthesized to mimic the pharmacological effects.<sup>3</sup> Clonazolam, etizolam, and flubromazolam are three designer benzodiazepines that have recently been observed in South Carolina casework. These drugs are marketed online as “research chemicals” and “not for human consumption.” Given the increase of designer benzodiazepines being shipped to the United States with very similar molecular formulas and structures to already scheduled benzodiazepines, there is a pressing need for confirmatory methods that can differentiate between analogous compounds.

Samples (1.0mL) were prepared for analysis using Solid Phase Extraction (SPE) with an elution solvent of ethyl acetate containing 3% ammonium hydroxide on Clean Screen<sup>®</sup> 10mL DAU columns. Chromatographic separation was achieved on a Liquid Chromatograph Tandem Mass Spectrometer (LC-MS/MS) system (Agilent<sup>®</sup> Technologies<sup>®</sup> 1290 LC coupled with an Agilent<sup>®</sup> Technologies<sup>®</sup> 6430 triple quad mass spectrometer) using positive electrospray ionization and dynamic multiple reaction monitoring mode with a UCT Selectra<sup>®</sup> DA column (50mm x 2.1mm, 3 $\mu$ m). Solvent A was 0.1% formic acid in Dionized (DI) water and solvent B was 0.1% formic acid in methanol. Mobile phase was introduced in a gradient programmed with 35% B, isocratic for one minute that was increased to 70% B over 7.5 minutes, increased again to 95% B for 1.6 minutes before the gradient was returned to the initial conditions and held for the remaining 1.8 minutes. The flow was set to 0.4mL/min with a total run time of 11 minutes.

The method was developed to be a sensitive assay with optimal run time. The following studies were all conducted in concurrence with the Scientific Working Group in Toxicology (SWGTOX) guidelines: bias, precision, calibration, dilution integrity, carryover, limit of detection, limit of quantification, and stability. Interference and ionization suppression/enhancement studies are currently in progress. The designer benzodiazepines had a defined limit of quantitation at 10ng/mL and limit of detection at 5ng/mL. The method was stable up to 72 hours and free from carryover at 7.5 times the highest calibrator. For the three designer benzodiazepines, within-run precision ranged from 3.6% to 8.2% for the Lower Limit Of Quantification (LLOQ) at 10ng/mL and 1.4% to 3.0% for the high control at 600ng/mL. For the low and high controls respectively, the between run precision ranged from 4.8% to 8.2%, and 2.7% to 4.7%, and the accuracy ranged from -5.7% to -0.56% and -2.2% to -0.6%.

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1. National Center for Biotechnology Information. *PubChem Compound Database*; CID=134664. [Internet]. Accessed July 10, 2018. <https://pubchem.ncbi.nlm.nih.gov/compound/134664>.
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### Benzodiazepines, Designer, LC/MS/MS



### E97 A Paper Analytical Device to Evaluate Illicit Drug Supply Chains

*Tracy-Lynn E. Lockwood, BS\*, South Bend, IN 46615; Marya Lieberman, PhD, University of Notre Dame, Notre Dame, IN 46556*

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**Learning Overview:** The goal of this presentation is to clearly demonstrate the different levels of the supply chain that illicit drugs take to make it to market. This includes the various hands passed through from cook to dealers to end user. It is believed that different drugs spend a different amount of time in the supply chain as they are diluted with fillers and other drugs such as fentanyl; this is believed to be how fentanyl came to be so prevalent in the market today. By using a paper-based analytical device designed to identify illicit drugs and various cutting agents to assess a simulated supply chain, we can evaluate how well this field device can track supply chains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new paper-based analytical device (idPAD) that can field test illicit drugs and identify various cutting agents and be used as a potential tool for law enforcement and first responders. The presumptive drug tests currently used by law enforcement officers in the field are often critiqued by the public due to high false positive rates and subjective interpretation. This paper analytical device uses less solid sample than current presumptive tests, costs less than the test pouches, and can be used in fewer than five minutes. Additionally, a portable light box ensures consistent lighting on the idPAD so the colors can be read accurately, regardless of the environment, which lowers user error. Current testing on the idPAD includes evaluating its ability to track various supply chains to better understand how and where these drugs are entering the market. Further understanding of the illicit drug supply line will provide law enforcement with information to hopefully lower the spread of drugs.

By combining 12 chemical color tests that target specific functional groups in illicit drugs as well as various cutting agents, a unique color-bar code is generated for each substance. When illicit drugs are being brought to market, there are dilutions with different cutting agents as they are passed through the hands of dealers, which can reveal useful information about the supply chain. The idPAD has been used to evaluate a simulated supply chain. The idPAD's ability to identify the illicit substances and the cutting agents used, and to evaluate how many people are creating distinguishable batches of products, will be discussed.

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#### Illicit Drug, Paper Analytical Device, Supply Chain

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## E98 Anabolic Androgenic Steroids Abuse: New Molecular Biomarkers

*Francesco Sessa, MS\*, Department of Forensic Pathology, University of Foggia, Foggia 71100, ITALY; Monica Salerno, MD, PhD, Department of Forensic Pathology, Foggia 71121, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY; Cristoforo Pomara, MD, PhD, Catania, ITALY*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of Anabolic Androgenic Steroids (AASs) assumptions, side effects, organ damages, and MicroRNAs (miRNAs) dysregulation. The first results obtained on this research hypothesis will be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a systematic review and meta-analysis on the miRNA dysregulation linked to the adverse effects of AASs use/abuse. This review also aims to suggest a new study hypothesis on the role of miRNAs as new molecular biomarkers of AASs use/abuse.

Currently, even if AASs abuse is clearly associated with a wide spectrum of side effects, frequently adolescents and athletes use a large group of synthetic derivatives of testosterone, both for aesthetic uses and for improving performances.

Nandrolone, testosterone, stanozolol, methandienone, and methenolone are the most frequently abused androgens and account for a major fraction of androgen abuse. One common factor between detecting each of these performance-enhancing drugs is that the traditional methods of drug testing were taken at a single point in time. An enhanced method of detection has been required to counter the increasingly sophisticated doping regimens and the ongoing development of new substances. For these reasons, the identification of new molecular biomarkers remains an ambitious target for the scientific community.

In the past few years, the development of microRNA (miRNA) technologies has become an essential part of research projects. Their role as potential molecular biomarkers is frequently investigated by the scientific community. The principal application of miRNA dosage is the characterization of cancer; moreover, miRNAs are frequently investigated as prognostic biomarkers, evaluating the disease evolution. More evidence suggests that miRNAs are deregulated in viral infections, nervous system disorders, cardiovascular disorders, muscular disorders, diabetes, and other diseases.

The research hypothesis of this presentation is a direct implication among drugs assumption, side effects, organ damages, and miRNAs dysregulation, with the goal of finding a direct link between AASs use/abuse and miRNA dysregulation. Moreover, with this proposal, the results obtained with the experimental model used will be presented.

Databases from 2000 to June 2017, including Medline®, Cochrane Central, Scopus®, Web of Science, Science Direct®, EMBASE, and Google® Scholar, were searched using the following keywords: miRNA, anabolic androgenic steroid, side/adverse effects, organs and systems (Cardiovascular system, reproductive system, central nervous system, liver, kidney, and skin). The main keywords were individually searched in association to each of the others.

Due to reviewing and analyzing each adverse effect caused by AASs use/abuse, a selection of miRNA signatures was performed. For example, a pivotal role on the activity of the cardiovascular system was played by myo-miRNAs; the up/under-expression could be important to generate adverse effects after AASs assumption. Following the research proposal, the dysregulation of the expression profiles of these miRNAs could suggest a direct action on tissues. Moreover, the same miRNAs could become the signatures of AASs abuse. Due to Real-Time Polymerase Chain Reaction (RT-PCR) technique, the miRNAs expression levels were evaluated on autopsy samples of young male deaths with toxicological positive tests for anabolic agents.

In conclusion, the identification of new tools for AASs use/abuse represents an important challenge for the scientific community. In the past few years, several studies highlighted the role of miRNAs as a highly accurate diagnostic tool. Moreover, miRNAs in serum are stable at room temperature and are resistant to freeze-thaw cycles; these characteristics highlight the important role of miRNAs in the future as new tools for the anti-doping war.

Knowledge regarding miRNA in human diseases related to AASs use/abuse may eventually lead to serum or tissue biomarkers with anti-doping utility. In this regard, there are major challenges, such as the need for careful validation of diagnostic miRNA candidates in well-annotated toxicological studies. The rapid progress in anti-doping technologies using miRNA-based strategies for discovered the drugs of abuse, such as AASs use/abuse, allow optimism for new approach definitions based on existing and emerging knowledge.

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### Anabolic Androgenic Steroids (AAS), miRNA, Adverse Effect



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### **E99 The Comparison of Entry Bullet Holes in Glass, Metal, and Wood With a Variance in Caliber, Distance, and Grain Weight**

*Mark A. Waldo, MFS\*, Santa Ana Police Department, Santa Ana, CA 92702; Ismail M. Sebetan, MD, PhD\*, National University, La Jolla, CA 92037-1011; Paul Stein, PhD\*, National University, La Jolla, CA 92037*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the differences in entry bullet holes in glass, metal, and wood substrates created by variables often encountered in shooting incidents: (1) different caliber ammunitions, (2) different grain weights, and (3) shooting distances.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the understanding of entry bullet holes and validating expert testimony regarding the origin and relationships of these in the reconstruction of shooting incidents.

This study hypothesized that a larger caliber bullet with a heavier grain weight would make a larger entry bullet hole, compared to a smaller caliber bullet with a lighter grain weight. It also hypothesized that a bullet fired at a closer distance would make a larger entry bullet hole than being fired at a further distance and one could expect significant differences in entry bullet holes depending on the nature of the target substrate. These possibilities were tested at an outdoor shooting range.

The following studies were conducted on three different surfaces (laminated glass, sheet metal, and plywood). The first study was conducted using a laminated glass as the target to simulate a vehicle windshield (24"x24" and 7mm thick). The second study was conducted using sheet metal as the target to simulate a metal vehicle quarter panel (24"x24" and 20 gauge). The third study was conducted using a plywood target (24"x24" and 1/2") to simulate a covering of an outdoor structure. There were three different shooting distances: near (4"), intermediate (24"), and distant range (48"). The shooting distances were measured from the muzzle of the handgun to the target surface. Six shots were fired for each caliber and grain weight: six shots for the distances of the metal and wood targets and only four shots for the glass target, due to the fragility of the glass. The entry holes were photographed with a Canon® EOS 70D camera and an American Board of Forensic Odontology (ABFO) metric scale. Camera settings were set manually according to the lighting at the time of shooting. The diameters of the entry holes were measured with a digital caliper and the data analyzed for statistical significance using the One-Way Analysis of Variance (ANOVA) statistical test and a p value of <0.05 to indicate significance.

The findings indicated that there was no statistical significance difference in the diameter of the entry hole in the targets, handgun caliber, grain weight, and firing distances used in this investigation. This research may dispel the often-held myths related to the tested variables and will provide crucial information for establishing the truth by supporting or refuting eyewitness or suspect testimony in the courtroom.

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#### **Shooting Distance, Bullet Entry Hole, Bullet Grain-Caliber**



## E100 An Investigation Into the Discriminating Potential of Different Techniques for the Analysis of Cosmetic Foundations

Jessica McFarland\*, North Wales, PA 19454; Thomas A. Brettell, PhD, Cedar Crest College, Allentown, PA 18104; Megan Zellner, MSFS, L'Oreal Cosmetics, Clark, NJ 07066; Lawrence Quarino, PhD, Cedar Crest College, Allentown, PA 18104

**Learning Overview:** After attending this presentation, attendees will have a better understanding of how cosmetic foundations can be discriminated using Raman microspectrophotometry, Attenuated Total Reflectance (ATR) infrared spectroscopy, and Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM/EDX).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a basis for the use of multiple techniques to distinguish between cosmetic foundation samples. All three techniques can be used without any sample preparation, which makes them quick and easy and allows for a minimal risk of contaminating or diluting the sample.

Cosmetics, such as foundations, are easily transferrable personal care products that can leave evidence at a crime scene. A smear could be left behind on anything that comes into contact with a victim or assailant wearing a cosmetic. This transferred smear could then be used to corroborate accounts of events, if the evidence can be compared with a known sample. Little research has been done in the way of analyzing foundation samples in a forensic context, although it is unclear as to why.<sup>1</sup> There is a large variety of components in cosmetic foundations, and these vary between manufacturers, foundation types, and foundation colors.<sup>2</sup> If a technique, or combination of techniques, could determine the differences, then it could be used to differentiate between foundations.

In this study, 34 cosmetic foundation samples from a variety of manufacturers and types were analyzed. For all techniques, the samples were analyzed with little sample preparation. For Raman analysis, each sample was placed directly onto a glass slide for analysis. Three different locations on the sample were scanned 16 times. The spectra for each sample were determined to be reproducible. Each sample spectrum was then compared to every other sample spectrum in pairs. Of the 561 pairs, 90.55% could be discriminated.

Using ATR, each sample was analyzed in triplicate. Each spectrum was obtained using 32 scans at a resolution of  $4\text{cm}^{-1}$ , with a background taken prior to each collection. The spectra for each sample were determined to be reproducible. Each sample spectrum was then compared to every other sample spectrum in pairs. Of the 210 pairs, 95.23% could be discriminated.

An SEM fitted with an iXRF<sup>®</sup> EDX unit was used to collect elemental information about each of the samples. Each sample was placed onto a piece of carbon tape before being analyzed. Every sample was analyzed at 200 times magnification in three different areas. A 20-kV beam with a 4.4 spot size was used. The concentration of elements in each sample was then analyzed with Principal Component Analysis (PCA) using BioVinci (version 1.1.3). Principal components 1, 2, and 3 were found to account for 40.60%, 22.55%, and 14.76% of the variance, respectively. Visual examination of 3D plots showed that replicates of each sample clustered together and could be distinguished from others.

In conclusion, using a combination of Raman microspectrophotometry, ATR infrared spectroscopy, and SEM/EDX shows the potential to differentiate between 34 cosmetic foundation samples. The use of non-destructive techniques with little to no sample preparation allows for the preservation of samples. The combination of the three techniques provides the forensic scientist a method for comparison of cosmetic foundation samples that are complimentary, easy to use, and sensitive.

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### Trace Evidence, Raman Spectroscopy, Cosmetic Foundations





**E101 Strengthening the Evaluation of Forensic Glass Evidence Using a Likelihood Ratio Approach**

*Ruthmara Corzo\**, Florida International University, Miami, FL 33199; *Jose R. Almirall, PhD*, Florida International University, Miami, FL 33199

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**THIS ABSTRACT WAS NOT PRESENTED.**

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## E102 Preparation of Molecularly Imprinted Polymers for the Extraction of Azo and Anthraquinone Dyes From Diesel Fuel

*Antonija Glavac, BSc, Zagreb, CROATIA; Hrvoje Sarajlija, PhD\*, Zagreb, Croatia, CROATIA; Ivana Bacic, PhD, Forensic Science Centre "Ivan Vucetic," Zagreb 10000, CROATIA; Dragana Mutavdic Pavlovic, PhD, Zagreb, CROATIA; Gordana Matijašić, PhD, Zagreb, CROATIA*

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**Learning Overview:** The goal of this presentation is to demonstrate preparation, characterization, and efficiency of Molecularly Imprinted Polymers (MIPs) designed to extract azo and anthraquinone dyes from complex matrices such as diesel fuel. MIPs are prepared for the Solid Phase Extraction (SPE) to be a faster, simpler, and more accurate way for the determination of the presence of these dyes in "laundered" fuel, even when they are present at very low concentrations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into how novel polymeric materials, prepared in a laboratory in an economical and fast way, and used as a sample pretreatment can improve detection of the desired analytes and thus provide valuable forensic evidence.

Fuel consumption is steadily rising, which generates substantial profits for the oil industry, but also causes fuel prices to rise. Except, the economic factors, local laws, and regulations also determine the level of fuel tax, which differs from country to country and depends on its use. In Croatia, fuels are distinguished by use for transport vehicles, heating fuel, and fuel for propulsion of agricultural machines. Blue diesel, intended for use in agriculture, fishing, and aquaculture, has a taxable amount of 0.0 and is classified as a tax-reduced energy source, unlike tax-fueled diesel, for which the price is higher for even 0.4 €L. In order to distinguish fuels depending on use and to prevent misuse, fuels are marked with a dye and a marker. The most commonly used marker in the European Union is Solvent Yellow 124 (SY124), the application of which is legally prescribed in Croatia. This marker is not visible to the naked eye, but only by extraction with hydrochloric or sulfuric acid, and the fuel is also labeled with visible colorants. Colorant used for labeling blue diesel in Croatia is Solvent Blue 35 (SB35), and Solvent Red 19 (SR19) is used for labeling heating oil. The fuel quantity is not legally prescribed, as opposed to the SY124 marker, which is added to the fuel in the amount of 6.0mg/L to 9.0mg/L. A big difference in the tax on such fuels, unlike fuel for transportation and its ever-increasing cost, leads to misuse. The illegal practice of removing fuel differentiation components and reselling it at a higher price is called fuel "laundering." Fuel "laundering" occurs by adsorption of markers and colorants from fuel with widely available materials and chemicals. Losses for the state are measured in the millions. Except for the state, users of such fuel are also damaged, and the substantial damage is made to the environment because of the waste, which is disposed without proper care.

As marker SY124 is not noticeable in fuel to the naked eye, intense work is conducted on the development of analytical procedures for its detection, especially in "laundered" fuel where its concentrations are extremely low. In addition to the SY124 marker, it is also important to prove the presence of blue and red dyes such as SB35 and SR19. For this purpose, MIP syntheses were performed using 4-vinylpyridine, methacrylic acid, or hydroxyethyl methacrylate as functional monomers, ethylene glycol dimethacrylate as a crosslinker, 2,2'-Azobis(2-methylpropionitrile) (AIBN) as an initiator, and acetonitrile as a porogen. Template molecules were dyes SY124, SR19, and SB35 or combinations thereof. Various methods such as Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), the Brunauer-Emmett-Teller surface analysis method, and Evolved Gas Analysis-Mass Spectrometry (EGA-MS) have been used to characterize the MIPs. The efficiency of the prepared printing polymers was monitored by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) and compared to commercially available SPE columns. The obtained results indicate that MIPs have a possible application in the daily problem of fuel "laundering."

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### Solvent Yellow 124, Fuel Laundering, Molecularly Imprinted Polymers



### E103 Self-Organizing Maps to Analyze the Relationship of Ignitable Liquids and Substrates

Nicholas A. Thurn\*, University of Central Florida, Orlando, FL 32826; Mary R. Williams, MS, National Center for Forensic Science, Orlando, FL 32816-2367; Michael E. Sigman, PhD, University of Central Florida, Orlando, FL 32816

**Learning Overview:** After attending this presentation, attendees will understand how the components of substrate backgrounds relate to components of the American Society of Testing Materials Standard Test Method for Ignitable Liquid Residues in Extracts from Fire Debris Samples by Gas Chromatography/Mass Spectrometry (ASTM E1618-14) ignitable liquid classes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an easy-to-visualize graphical representation of the relationship between two reference databases to better inform the analyst of where problematic class overlap can occur and types of substrate background that may interfere with the interpretation of casework samples.

Fire debris samples are currently analyzed according to ASTM E1618-14, which makes use of the total ion chromatogram, extracted ion chromatogram, and target compounds. Ignitable Liquid (IL) residues are classified as one of the following seven classes given by ASTM E1618-14: gasoline, petroleum distillate, isoparaffinic, aromatic, naphthenic-paraffinic, normal alkane, and oxygenate. The chromatographic profiles and the relative presence of the major compound types are used to determine which classes the IL residue falls into. Any ignitable liquids that do not fall into these classes are designated as miscellaneous and have characteristics of multiple classes. Even with the guidelines provided by ASTM E1618-14, determining the presence of IL residues is still a time-consuming manual pattern recognition process for the analyst and is highly subjective in nature. To counteract this, automated chemometric methods that use the Total Ion Spectrum (TIS) are being developed. The TIS is the time-averaged mass spectrum across the total chromatographic profile and allows for comparison within or between labs due to the absence of the retention time variation. This work makes use of the Extracted Ion Spectrum (EIS), a subset of the TIS, which is generated using the ions in Table 2 of ASTM E1618-14. These ions are representative of the major compound types present in each of the ASTM classes and include: normal alkanes, branched alkanes, cycloalkanes, aromatics, polynuclear aromatics, ketones, and oxygenates.

Self-Organizing Maps (SOM) are a type of artificial neural network used for the visualization of large datasets and can be easier to visually interpret than principal component analysis for complex data. In a previous study, it has been shown that IL group according to their ASTM class designations using the EIS of ASTM ions and the SOM. This work expands on previous work and uses SOM to examine the relationship of substrates and IL from the National Center for Forensic Science (NCFS) Substrate and Ignitable Liquid Reference Collection (ILRC) databases. Substrates grouped in the center of the map with the ASTM classes surrounding them. However, significant overlap was observed between substrates and the oxygenate class. Substrates were labeled according to their major polymer type and whether they are derived from wood or non-wood materials. Ground truth samples were also projected onto the map to examine the potential of the trained map to classify fire debris samples. There was a reasonable trend observed for the position of the ground truth samples on the map with respect to the ratios of the individual components used to create them. This study demonstrates the power of unsupervised machine learning methods to be used as a quick screening tool in forensic science and allows the analyst to qualitatively assess the class or components of a casework sample.

#### Fire Debris, Chemometrics, Machine Learning



### E104 An Application for Forensic Analysis: The Discrimination of Fibers Using a Trace Organic Additive and Pyrolyzate Marker

Chikako Takei, BioChromato Inc, Fujisawa-shi, Kanagawa-ken 251-0053, JAPAN; Kenichi Yoshizawa, MPharm\*, BioChromato, Inc, Fujisawa 251-0043, JAPAN

**Learning Overview:** After attending this presentation, attendees will understand the value of a Thermal Desorption and Pyrolysis device combined with Direct Analysis in Real Time Mass Spectrometry (TDP/DART®-MS) for the rapid discrimination of polymer material products, such as cloths, fibers, and containers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining how TDP/DART®-MS can be effectively applied as an identification and screening technique for tracing evidence.

Scientific examination of miscellaneous evidence collected at the crime scene is required to extract information during a criminal investigation. There are two methods of approaching physical evidence, namely, identification and comparison.<sup>1</sup> The former involves surveying and recording the substances that make up the evidence. In the latter, the same inspection is applied to a specimen of unknown origin (suspected sample) found at the scene of the crime and an apparent specimen (controlled sample) derived from or near the suspect. Subsequently, an analysis is performed using test results to ascertain whether the two are from the same source. The comparison process uses a combination of numerous methods, including observing features and appearance, measuring physical properties, and analyzing chemical compounds. For chemical analysis, comparison of trace compounds is sometimes more effective than the main compound.<sup>2</sup>

The DART® ion source is one of the ambient ionization methods reported by Robert B. Cody et al. in 2005.<sup>3</sup> It is excellent in versatility and speed, as ionization occurs easily by holding the sample over an ion source. However, when a product consisting of a polymer material, such as cloth or plastic, is to be analyzed, it is difficult to observe the peak of the target compounds. Therefore, a TDP process was developed for DART®-MS, which consists of a device capable of directly heating the sample from room temperature to 600°C. It has also been established as being usable for the identification of additives and polymer matrices in polymer material products. In this presentation, it is shown that the TDP/DART®-MS method can be used for both identification and comparison of polymer material products by using the analysis results of various polymer materials.<sup>4</sup>

In this study, several types of cloth fibers were used as samples. Mass spectra were obtained by a quadrupole Time-Of-Flight (qTOF) mass spectrometer equipped with a DART® ion source and a TDP unit. The TDP unit was mounted between the DART® ion source and the mass spectrometer. Mass spectra were measured in the positive-ion mode as the samples were heated from room temperature to 600°C. Analysis results of fibers using TDP/DART®-MS, namely, organic additives contained in fibers and pyrolysis products of polymer matrix, were detected at the thermal desorption region and pyrolysis region, respectively. It was confirmed that this analysis method is useful for the identification of polymer materials by using the pyrolysis products of polymer matrices as a marker. Additionally, this study established that this analysis can be used for comparison by using both organic additives and pyrolysis products of these samples as a marker. Thus, the combination of conventional methods and TDP/DART®-MS could contribute to the differentiating of evidence in criminal investigations.

#### Reference(s):

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3. R.B. Cody, J.A. Laramée & H.D. Durst. Versatile New Ion Source for the Analysis of Materials in Open Air Under Ambient Conditions *Anal. Chem.*, 77, 2297-2302 (2005).
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#### Discrimination of Fibers, TDP/DART®-MS, Trace Organic Additive



### E105 Validation of New Test Media for Gunshot Residue Visualization in Distance Determination Methods

Steven J. Little, BS\*, Marshall University, Huntington, WV 25701; Jessica Coudriet, BS, North Carolina State Crime Laboratory, Raleigh, NC 27603; Jessica Pappas, MS, North Carolina State Crime Laboratory, Raleigh, NC 27603; Catherine G. Rushton, EdD, Marshall University Forensic Science Program, Huntington, WV 25701; Lauren L. Richards-Waugh, PhD, Marshall University Forensic Science Program, Huntington, WV 25701

**Learning Overview:** After attending this presentation, attendees will better understand the advancements in, as well as limitations of, contemporary substrates used to perform chemical analyses in gunshot distance determinations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by validating the capabilities of modern test media to replace the dwindling supply of conventional resources, as well as demonstrating the efficacy, or lack thereof, of the new materials when integrated into proven laboratory methodology.

Considering the staggering number of violent crimes committed using firearms in the United States, it is crucial for analysts to have a reliable means of evaluating and reconstructing a gunshot incident.<sup>1</sup> The estimation of muzzle-to-target distances is commonly inferred by measuring the diameter of the nitrite residue pattern transferred from the victim's clothing onto desensitized photographic paper via the Modified Griess Test (MGT), then comparing that measurement to a range of laboratory-generated standards. However, with the proliferation of digital photography, inkjet photo papers have been examined as a replacement to their increasingly costly and scarce predecessor with remarkable success.<sup>2,3</sup>

The goal of this study was twofold: (1) validate an inkjet photo paper to be incorporated into the Standard Operating Procedure (SOP) for the North Carolina State Crime Laboratory, and (2) evaluate the potential of the paper to retain adequate lead residues following Modified Griess processing to be revealed using an application of Sodium Rhodizonate.<sup>4</sup> The goal of the latter parameter was to identify a means of reinforcing the results obtained through the traditionally accepted methodology.

During the MGT, a hot clothing iron is pressed onto an assembly comprised of a piece of gauze saturated with a 15% acetic acid solution and the sample material placed face down on the emulsion side of photo paper. The paper was previously treated with a sulfanilic acid and alpha-naphthol solution. The acetic acid is vaporized and penetrates the sample material, inducing a chromophoric reaction on the photo paper indicating the presence of nitrite residues. It was the secondary intention of this study to confirm the incidental transfer of lead residues to the photo paper during MGT processing. The Sodium Rhodizonate test could then be performed on the photo paper to reveal the presence of lead residues that may have been displaced by the MGT. The fundamental mechanism driving the movement of lead residues is similar to that of the Bashinski Transfer method, a previously verified procedure among firearms examiners.<sup>4</sup> Filter paper saturated with acetic acid is placed over a bullet hole on the victim's clothing, ironed until dry to attract the lead, and subsequently treated with Sodium Rhodizonate. If lead residues adhered to the photo paper in a similar manner during the MGT, the paper could be treated with Sodium Rhodizonate alongside the other materials, and the results used to supplement any findings using the Bashinski method.

The inkjet paper performed comparably to the desensitized paper without any alterations to the established Modified Griess procedure, noting that the papers with a glossy or semi-gloss finish were substantially better suited for this analysis. It was not possible, though, to observe lead residues on the photo paper to a degree that would satisfy evidentiary standards or outperform the recognized methodology.

#### Reference(s):

- <sup>1</sup> *Crime in the United States: 2016*. Uniform Crime Reporting, Federal Bureau of Investigation, last modified April 12, 2018, <https://ucr.fbi.gov/crime-in-the-u.s/2016/crime-in-the-u.s.-2016>.
- <sup>2</sup> Kathleen A. Hafeli. *Investigating a Substitution for Photographic Paper When Performing the Modified Griess Test for Gunshot Residue Analysis*. MS thesis, California State University, Los Angeles, 2012, 23-40.
- <sup>3</sup> Philip A. Hess, BS, MS, and Leslie L. Poole, BS, D-ABC. The Validation of Inkjet Photographic Paper for Use with the Modified Griess Test. *AFTE Journal* 37, no. 3 (Summer 2005): 213-223.
- <sup>4</sup> Firearms Technical Leader. Technical Procedure for Distance Determination. *North Carolina State Crime Laboratory—Firearms Unit*, version 7 (September 2017): 3-9.

#### Firearms Examination, Gunshot Residue, Distance Determination



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### E106 A Comparison of Gunshot Residue (GSR) Visualization With Alternate Light Sources (ALS) and Infrared (IR)

*Mark Vecellio, MFS\*, Methodist University, Fayetteville, NC 28311; Alycia M. Smentkiewicz\*, Methodist University, Garner, NC 27529; Sarah V. Morello\*, Raeford, NC 28376; Patrick M. Wright\*, Saint Pauls, NC 28384*

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**Learning Overview:** After attending this presentation, attendees will understand how ALS and IR light is used to locate GSR on dark-colored substrates and recognize the need for additional research concerning the detection of GSR on painted surfaces.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting a better understanding of the capabilities of ALS and IR in visualizing GSR on dark-colored surfaces and will also help attendees understand the need for additional research on how paint may affect visualization of GSR.

Initial detection of GSR at a crime scene, even if presumptive in nature, may aid investigators in better understanding shooting scenes and can be very valuable during a variety of investigative tasks, particularly interviews and evidence collection. GSR can be observed on many surfaces; however, dark-colored surfaces often obscure it. This study examines two methods of presumptively detecting the presence of GSR on dark-colored surfaces, both ALS and IR.

Four types of ammunition (.22, .223, 9mm, and .45) were fired five times each from a distance of six inches into two substrates: (1) black, cotton tee-shirt samples, and (2) drywall samples painted with Valspar® Black Gloss Latex Enamel paint. The samples were later observed using Sirchie TMX (395nm and 450nm) and Sirchie Megamax (530nm) ALS light sources with appropriate orange and red barrier filters, and a Fuji XT1 IR camera with IR 695 and IR 830 filters was utilized, in conjunction with 100-watt incandescent light sources, to observe the samples with IR. All samples were viewed with the same ALS and IR equipment prior to shooting for control purposes. No particles were detected on any of the samples prior to shooting.

GSR was visualized on all 20 cotton tee-shirt fabric samples using both IR (both filters) and ALS 450nm. ALS 530nm allowed visualization of GSR on 14 of the 20 samples, while ALS 395nm allowed visualization of GSR on seven of the 20 samples. GSR was not identified on any of the painted drywall samples using any of the light sources.

The GSR observed with IR on cotton fabric was located in close proximity to the bullet defects, while the particles observed with ALS (all wavelengths) were scattered and found up to four inches away from the bullet defects. Though these methods should only be applied as search tools, the GSR identified with IR could be easily interpreted due to its contiguous presence around the circumference of the bullet holes. However, the scattered particles detected with ALS could cause interpretation difficulties when other trace particles are present, thus potentially minimizing some of the immediate interpretative value of the evidence.

The inability to visualize GSR on the painted drywall using either method is significant. Additional research could analyze how other paints may affect visualization and seek alternative visualization methods.

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#### GSR, Infrared, ALS



**E107 The Detection and Identification of Organic Gunshot Residue (OGSR) Via Fluorescence Mapping and Raman Spectroscopy**

*Shelby R. Kandasammy, BS\*, State University of New York at Albany, Albany, NY 12222; Alexander Rzhetskii, PhD, Thermo Scientific, Hull, MA 02045; Igor K. Lednev, PhD, State University of New York at Albany, Albany, NY 12222*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### E108 Laser-Induced Breakdown Spectroscopy (LIBS) as a Rapid Detection Technique for Gunshot Residue (GSR)

Korina Menking-Hoggatt, MSc\*, Morgantown, WV 26501

**Learning Overview:** The goal of this presentation is to inform attendees of a rapid technique for the detection of Gunshot Residue (GSR) using LIBS and the performance measures associated with the method.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees that LIBS can decrease turnaround time by providing practitioners with a rapid, reliable screening test for GSR detection on hands.

The detection of GSR is crucial during the investigation of firearm-related crimes, and is, therefore, a commonly submitted form of evidence to forensic laboratories. The United States National Crime Victimization Survey (NCVS) most recent data reported 480,000 firearm-related crimes in 2016, and a report by Project FORESIGHT for the 2015-2016 fiscal year found the average turnaround time for GSR to be between 19 and 89 days and cost between \$800 to \$3,500 per forensic report.<sup>1,2</sup> The current American Society for Testing and Materials (ASTM) standard requires the identification of characteristic elements (typically lead, barium, and antimony) using Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDS).<sup>3</sup> Although the SEM/EDS method is reliable, it is also costly and time consuming. The goal of this study is to develop a reliable screening method capable of detecting GSR in just a few minutes with minimal destruction of the sample, high specificity, and sensitivity. LIBS is proposed for GSR detection using a universal sampling method compatible with current practice (SEM/EDS). The advantages of this method are the simultaneous multi-element capability, selectivity, sensitivity, versatility, and portability.

A validation set of 296 samples originating from 60 non-shooters and 56 shooters was used to evaluate the accuracy of the method. The non-shooter specimens were collected from at least one of the hands of each individual using the standard carbon adhesive tabs. The sample set from known shooters was collected using four GSR separate stubs from the hands of each individual after firing a pistol or a revolver (right back, right palm, left back, and left palm).

The research presented here developed two different LIBS acquisition methods, one ablating a line of 7mm by 100 $\mu$ m at the GSR stub surface, and another shooting the laser in a 5 by 5 grid of 100 $\mu$ m diameter spots. The grid method allowed for the addition of chemical mapping and spatial information to determine the location of the GSR species on the stub. Both methods left more than 99.9% of the carbon stub intact for further confirmatory testing. Data pre-processing included background subtraction, peak identification, and peak integration. Box plots and principal component analysis were used for exploratory analysis of the data. A positive threshold/result was established when the signal of the analyte was above the limit of detection and exceeded the non-shooter background signal by at least three standard deviations. Alternatively, discriminant analysis was used as a classification tool to group unknowns into shooter or non-shooters categories. The line scanning method showed a true positive rate (sensitivity) of 73%–86%, a true negative rate (specificity) of 97%–100%, and an overall accuracy of 87%–90%, depending on the statistical classification method. The grid method showed a true positive rate (sensitivity) of 73%–100%, a true negative rate (specificity) of 96%–100%, and an overall accuracy of 82%–100%.

The results demonstrated that LIBS is as a rapid, reliable technique with high sensitivity and specificity for GSR detection. The incorporation of a rapid screening method into the current protocol of GSR examination could minimize unnecessary confirmatory analysis of negative samples, reducing costs and offering a more streamlined and efficient management of casework. Moreover, LIBS will provide complementary information to SEM/EDS and increase confidence in the interpretation of the evidence.

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2. *Project FORESIGHT Annual Report, 2015-2016*. West Virginia University, College of Business and Economics combined with the Forensic Science Initiative. 2016.
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#### Gunshot Residue (GSR), Rapid Detection, LIBS





**E109 A Review of Forensic Analysis of Phenolphthalein in Bribery Cases**

*Geetanjali Sachdeva, MSc\*, Forensic Science Lab, Haryana, Kurukshetra 136118, INDIA*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### E110 Violence Against Health Workers in Turkey

*Inci Y. Tezbasan, BSc\*, Uskudar University, Istanbul 34662, TURKEY; Ümit Ertem, MSc, Üsküdar University, Istanbul 34662, TURKEY; Sevil Atasoy, PhD, Uskudar Üniversitesi Adli Bilimler Enstitüsü, Istanbul, TURKEY*

**Learning Overview:** The goals of this presentation are: (1) to present some featured cases that appeared widely in the national media, (2) to present statistical results regarding violent attacks by patient or relatives during medical service, and (3) to discuss possible intervention techniques in Turkey.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing some nationally featured cases, statistical results regarding violent attacks during medical service, and possible intervention techniques in Turkey.

Violence is defined by the World Health Organization as “the intentional use of physical force or power, threatened or actual, against oneself, another person, or against a group or community, which either results in or has a high likelihood of resulting in injury, death, psychological harm, maldevelopment, or deprivation,” although the group acknowledges that the inclusion of “the use of power” phrase in its definition expands on the conventional understanding of the word.

In health institutions, violence is defined as “the case consisting of the threatening behavior, oral threat, economical misuse, physical assault and sexual assault risky for the health care workers, caused by the patient, the patient’s relatives, or any individual.” The health care workers are in communication with the patient and his/her relatives, having socio-cultural characteristics that are different from each other due to their professions. It affects the treatment’s process, the patient’s satisfaction of the health care worker at his/her service, and the hospital’s reputation.

Working at a health care institution is riskier than working in other places in regard to violence. It is particularly observed that the frequency of oral violence is higher than physical violence in health care institutions. There are also various risks in the fields in which health care services are offered as there are in all fields in which the human life continues. It is quite important to solve these risks previously, to determine what should be done, and how risk management should be ensured when it is performed. Therefore, various means were needed in order to manage immediate cases, a number of the stimulating systems, and the interference codes aimed at them. Color codes are one of the methods that are most commonly used worldwide. These codes announce/notify hospital workers in cases of risk when communication is allowed, providing a short, clear message that provides time for the right interference, prevents the occurrence of panic, assists in enabling emergency preparedness, and provides security for patients and workers in the institution.

The “white code,” used to provide security for patients and workers, is the emergency management method aimed at preventing violence to workers in hospitals. For the code system to work well, it is extremely important to provide training for the health care workers regarding the system and to have regular practices focused on these codes.

The rise in violence in recent years and gynecologists being the primary target will be interpreted and the proper use of the “white code” and the follow-up procedures will be discussed.

#### Health Workers, White Code, Violence Prevention



### **E111 The Role of Human Rights as a Reason for Refugee Status and Immigration Requests: A Retrospective Analysis of Southern Italy**

*Isabella Aquila, MD\*, Institute of Legal Medicine, Catanzaro 88100, ITALY; Matteo A. Sacco, MD\*, Chair of Legal Medicine, University of Catanzaro, Catanzaro 88100, ITALY; Elena Sacco, Luca & Partners, Crotona, ITALY; Piero Lucà, Lucà & Partners, Crotona, ITALY; Fabio Lucà, Lucà & Partners, Crotona, ITALY; Santo Gratteri, MD, Viale Europa, Germaneto, Catanzaro 88100, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY*

**Learning Overview:** After attending this presentation, attendees will understand the more common reasons why migrants leave their countries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the need to promote the defense of human rights.

Immigration is a phenomenon of great social impact. In recent years, there was an increase in disembarkations in the Mediterranean Sea. In 2017, according to United Nations High Commissioner for Refugees (UNHCR) data, approximately 110,000 migrants landed in Italy. In recent years, forensic sciences have also been interested in this phenomenon. In 2017, more than 2,300 immigrants died.<sup>1</sup> This was related to the risk of drowning, dehydration, and dangers associated with travel. Despite the spread of the phenomenon, the reasons for escape from their native countries are not always known. The goal of this study is to assess the reasons for legal requests of refugee status.

The study was carried out with the collaboration of a law firm in southern Italy that analyzed the rights of approximately 1,500 immigrants. A retrospective analysis of the testimonies of 500 immigrants disembarked in southern Italy was performed. The data were collected from interviews carried out and performed with a native speaker. The interviewees were asked to explain the reasons why they had left their own country. The data were then classified according to sex, country, and reason for requesting a residence permit for every immigrant. Data was compared and analyzed.

The retrospective study revealed that 98% of the questioned people were male and all came from Asia or Africa. Regarding geographical origin, 289 (57.8%) came from Pakistan, 42 from Bangladesh (8.4%), 42 from Nigeria (8.4%), 37 from the Gambia (7.4%), 37 from Senegal (7.4%), 16 from Ghana (3.2%), 12 from Mali (2.4%), 8 from the Ivory Coast (1.6%), 6 from Guinea (1.2%), 3 from Guinea Bissau (0.6%), 2 from Niger and Afghanistan (0.08%), and 1 from Sierra Leone, Liberia, Burkina Faso, Togo (0.08%). Approximately 40% provided evidence or documents regarding their origins.

The main reason for escape was terrorism (30.6%), followed by religious (14.2%) and political persecutions (13.6%). Among the remaining reasons were unjust judicial persecutions (11%), personal persecutions (9%), homosexuality (6.8%), family persecutions (6%), and poverty (3.8%). The remaining 5% of cases of escape were due to female circumcision, mistreatment, sexual violence, ethnic discrimination, war, or illness.

From this investigation, terrorism was the reason for escape, especially from Pakistan, and the victims named specific organizations. The migrants describe many attacks, forced disappearances, systematic homicides involving family members, witnessed by photos, threat letters, newspaper articles, and images about autopsies or funerals of family members.

Regarding religious persecutions, most reported attacks on religious minorities, such as Ahmad, Shiite, Hindu, Christians, or violence committed by members of religious sects. Religious persecutions were the first reason for leaving from Nigeria and Senegal but were also present in Gambia and Ghana.

With regard to political attacks, migrants described many episodes of violence by police or opponents belonging to terrorist groups during demonstrations. Political persecution was the first reason for escape from Bangladesh.

Unfair justice persecutions, due to episodes of repression of the judiciary or fear of unjust detention, torture, or death sentences, were the first reasons for escaping from Gambia and Ghana.

Homosexuality was considered a crime in nearly all countries examined, and nearly all of the homosexual interviewees said that they suffered threats, personal injuries, torture, and the death penalty.

Female circumcision was a relevant reason for escaping, especially from Africa, particularly in Senegal, Nigeria, and the Gambia. In fact, not only women but also men who opposed this practice were forced to escape, as they were condemned by their communities.

This study revealed that less than 4% reported poverty as a reason for escaping. Therefore, the serious violation of human rights in these countries would be the main cause of request for a residence permit. Although only 40% of interviewees provided evidence, their statements were significant for understanding the need to promote the defense of human rights in these countries. This study offers evidence of the importance of epidemiological data regarding the phenomenon of immigration in order to find new solutions for migratory flows and to improve assistance to refugees.

#### **Reference(s):**

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#### **Forensic Science, Human Rights, Immigration**



## E112 Perceptions of Task Relevance in Forensic Science: A Survey of Forensic Analysts

Brett O. Gardner, PhD\*, University of Virginia, Charlottesville, VA; Sharon Kelley, PhD, Institute of Law, Psychiatry, and Public Policy, Charlottesville, VA 22903; Daniel C. Murrie, PhD, Institute of Law, Psychiatry, and Public Policy, Charlottesville, VA 22908; Kellyn Blaisdell, BA, University of Virginia, Charlottesville, VA 22902

**Learning Overview:** The goal of this presentation is to educate attendees about predominant beliefs among forensic analysts regarding the relevance of information types when conducting forensic analyses and to demonstrate how such beliefs are integral to managing contextual bias.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reviewing the literature on contextual effects in forensic analyses and providing results of the first empirical attempt to discover what forensic analysts from wide-ranging disciplines believe to be task-relevant to their scientific analyses. This presentation will also discuss how results can shape future research, policy, and professional practice.

Forensic analysts are often exposed to wide-ranging contextual information (e.g., suspect criminal history) regarding cases as they complete analyses. At the same time, research across forensic science disciplines has shown that irrelevant contextual information can bias analyses, even though analysts are generally unaware that such information is influencing their decisions.<sup>1-3</sup> Based on this body of research, the National Commission on Forensic Science reported that “forensic science service providers should rely solely on *task-relevant* information when performing forensic analyses.”<sup>4</sup>

Scholars, government agencies, and national authorities increasingly caution courts about the effects of contextual bias on experts and have called for laboratories to implement context management procedures.<sup>5</sup> However, no research has examined what types of information forensic analysts consider to be task-relevant and task-irrelevant. This distinction is necessary to help implement recommended procedures that limit exposure to task-irrelevant information.

The present study surveyed forensic analysts regarding their opinions of what types of information commonly contained in evidence submission forms are “essential” versus “irrelevant” to the analysis of forensic evidence. Understanding what information forensic analysts consider to be essential versus irrelevant, and whether there is consensus among examiners, may play an important role in informing efforts to minimize contextual bias.

In total, 183 practicing forensic analysts from wide-ranging disciplines completed a three-part survey at the outset of five training programs in the United States. In brief, the survey presented analysts with a list detailing 16 different types of information regarding case details (e.g., offense type, description of evidence), suspect details (e.g., suspect name, suspect criminal history), and victim details (e.g., victim race, victim name), most of which are commonly found on evidence submission forms.<sup>6</sup> Participants then described the importance of each type of information when performing their specific duties as a forensic analyst (i.e., “analyzing evidence in your discipline”) by indicating whether they believed such information was “Essential,” “Irrelevant,” or “Not essential, but you would review this if available.”

This presentation will provide detailed charts and statistics summarizing analysts’ perceptions according to their primary discipline. In certain disciplines, there was a lack of consensus regarding the relevance of some pieces of information. For example, analysts within forensic chemistry were equally divided (i.e., ≈33% endorsed each relevance category) regarding the relevance of knowing offense type when conducting analyses. Across all disciplines, some patterns emerged regarding the types of information that analysts most frequently agreed upon. Analysts were more likely to agree on the task relevance of information relating to a suspect or victim than they were to agree on the relevance of case information (e.g., method of evidence collection). Further, analysts were most likely to agree that information, particularly information regarding a suspect or victim, was *irrelevant* to their analyses rather than *essential*. Analysts within crime scene investigation appeared unique from other disciplines as such analysts were much more likely to describe wide-ranging types of information as *essential*.

Taken together, the current findings reveal a lack of consensus regarding the relevance of some types of information (e.g., offense type) to forensic analyses and a majority opinion that other types of information are irrelevant (e.g., victim ethnicity). Given human vulnerability to contextual bias, it is critical to determine which information is essential to forensic analyses and to limit exposure to extraneous information. The present study consequently reveals a need for further work to understand what information is thought to be task-relevant versus task-irrelevant. Such information is critical to informing future methods (e.g., context management procedures) to limit bias.

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### Task Relevance, Bias, Contextual Effects

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### E113 National Institute of Justice (NIJ) Initiatives and Partnerships to Advance the Forensic Sciences

*Jonathan G. McGrath, PhD\*, National Institute of Justice, Washington, DC 20531*

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**Learning Overview:** After attending this presentation, attendees will better understand the initiatives developed by the Department Of Justice (DOJ) and the NIJ to advance the forensic sciences at the federal, state, local, and tribal levels. Attendees will learn how coordinated efforts and new partnerships are providing resources to meet the needs identified by the forensic science stakeholder community. Attendees will also learn how to access these resources, including reports, webinars, and learning opportunities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying resources developed through the work of several forensic working groups and will provide important information relevant to forensic science practitioners, medical examiners and coroners, forensic science stakeholders, and students.

The DOJ recently conducted a needs-based assessment of forensic laboratories that included public crime laboratories with medical examiner and coroner offices as an overview of academic forensic science resources. The NIJ supported this effort to learn from state, local, and tribal stakeholders about forensic services provided across the United States. Along the way, the NIJ learned critical information that is serving to enhance existing programs and foster new initiatives and partnerships. This presentation will discuss the NIJ's activities to identify key priorities and to develop practical solutions to support the forensic science community.

This presentation will discuss the NIJ activities to support the work of several working groups, including for the recently established Forensic Laboratory Needs-Technology Working Group (FLN-TWG), to help focus resources to achieve actionable results and the Office of Justice Programs/Department of Health and Human Services Federal Medicolegal Death Investigation (MDI) working group. This presentation will discuss recent developments from partnerships with the Bureau of Justice Assistance (BJA), the Bureau of Justice Statistics (BJS), and the Office of Victims of Crime (OVC), including training and technical assistance opportunities. This presentation will highlight the NIJ's coordination with the DOJ Office of Justice Programs (OJP) partner agencies to coordinate activities that involve forensic science and the criminal justice system stakeholder community. The BJA is advancing public safety and public responses to the opioid crisis through the BJA Comprehensive Opioid Abuse Program. The BJS is developing survey tools to implement the Census of Publicly Funded Forensic Laboratories and the Census of Medical Examiner and Coroner Offices. The OVC has developed a Vicarious Trauma Toolkit and a Mass Violence Toolkit. This presentation will also impact the forensic science community by identifying resources developed through these working groups and that are available through the NIJ Forensic Technology Center of Excellence that is supporting the forensic science community to advance technology, share knowledge, and address challenges.

The NIJ is the research, development, and evaluation agency of the DOJ and is dedicated to improving knowledge and understanding of crime and justice issues through science. The NIJ provides objective and independent knowledge and tools to reduce crime and promote justice. The NIJ supports the DOJ's priorities on forensic science to improve capacities, increase coordination and collaboration at the Department, among Federal agencies, and with state, local, and tribal entities, and advance the reliability and sensitivity of forensic science testing. The OJP's mission is to increase public safety and improve the fair administration of justice across America through innovative leadership and programs.

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#### **Federal Initiatives, Partnerships, Resources**



### E114 Addressing the Challenge of Conducting Undergraduate Research in Forensic Science Academic Programs Using a Course-Based Undergraduate Research Experience (CURE)

Kimberly S. Koboжек, MS\*, Arizona State University New College, Phoenix, AZ 85069-7100; Pamela A. Marshall, PhD, Arizona State University West, Phoenix, AZ 85069-7100; Ken G. Sweat, PhD, Arizona State University West, Phoenix, AZ 85069-7100; Jennifer Broatch, PhD, Arizona State University, Glendale, AZ 85306-4908; Jennifer Foltz-Sweat, PhD, Arizona State University West, Phoenix, AZ 85069-7100; Anthony B. Falsetti, PhD, George Mason University, Fairfax, VA 22030

**Learning Overview:** After attending this presentation, attendees will understand how to develop and execute a course-based method to integrate research into undergraduate forensic science academic programs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating a modality for integrating research into an undergraduate forensic science academic program, as well as creating a potential pipeline for mutually beneficial collaborations between crime labs and forensic science academic programs.

There are inherent challenges when conducting any type of Science, Technology, Engineering, and Mathematics (STEM) research. Money, personnel, and time are just a few of the overarching issues that researchers face. Forensic science is both more interdisciplinary and applied than many other “traditional” STEM fields, so the task of implementing a robust research experience for undergraduate students to the field of forensic science research may face additional challenges. Traditionally, research at the university level has been the purview of graduate students and academic faculty; thus, research monies are awarded to these groups and research positions for undergraduates can be difficult to obtain. While many undergraduate STEM programs have encouraged participation in research, tenure track, or other research, faculty may not be performing research of interest to the undergraduate forensic science student. Thus, students typically participate in existing faculty members’ research programs, which are often not specifically focused on the forensic sciences.

Both the 2004 Technical Working Group for Education and Training in Forensic Science (TWGED) report and the 2009 National Academy of Sciences Report made it clear to the forensic science community that research is essential for the support and advancement of the forensic sciences.<sup>1,2</sup> If crime laboratories are increasingly placing a premium on applicants who have research experience in addition to an appropriate science degree, then graduate students would be more sought after versus an applicant with “only” a four-year science degree. However, many students choose not to continue in a graduate program. This deficiency of participation in graduate programs coupled with the reality that there are not as many specific forensic science graduate programs as there are other traditional STEM graduate programs in the United States leads to a dearth of research-trained forensic scientists. This situation creates a demand that is difficult to meet and places students, educators, and crime laboratories in a bind.

Employing a CURE is a method for both new and established undergraduate forensic science programs to incorporate research into their undergraduate curriculum. By using a CURE modality, undergraduate students can participate in authentic research and carry that experience with them as a unique skill set that crime laboratories and other forensic-based employers are seeking.

The development of a core CURE curriculum designed to be used by multiple disciplines was created by an interdisciplinary collaboration at Arizona State University funded by the National Science Foundation. This model curriculum was applied to the forensic sciences by investigating the persistence of latent forensic evidence. The core curriculum included document templates and teaching module outlines for instructors and students to use throughout the course. These materials covered the importance of research; what “good” research looks like; experimental design, execution, and analysis; and presentation of research via different manners—scientific poster, oral presentation, and/or publishable research paper.

Research is no longer the province of just graduate programs and tenure-track academics. The CURE can serve as a single-semester research experience or a year-long capstone project. Whatever the format, the CURE model is an innovative and unique opportunity for undergraduate students to perform research at the university level, gain valuable experience while still an undergraduate student, and potentially collaborate with practicing forensic scientists on current research and/or validation projects.

*This material is based upon work supported by the National Science Foundation. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.*

#### Reference(s)

1. U.S. Department of Justice, Office of Justice Programs, National Institute of Justice. Technical Working Group for Education and Training in Forensic Science (TWGED). *Education and Training in Forensic Science: A Guide for Forensic Science Laboratories, Educational Institutions, and Students*. Washington, DC, 2004. <https://www.ncjrs.gov/pdffiles1/nij/203099.pdf>.
2. Committee on Identifying the Needs of the Forensic Sciences Community, National Research Council. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC, 2009. <https://www.ncjrs.gov/pdffiles1/nij/grants/228091.pdf>.

#### Education, Undergraduate Research, Forensic Science



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### E115 The Use of Large-Scale Faux Crime Scenes and Cold Case Coursework to Teach Forensic Science in Social Science-Based Coursework

*Michelle Rippy\*, California State University-East Bay, Hayward, CA 94542*

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**Learning Overview:** After attending this session, attendees will be able to identify common issues with teaching science-based courses in a social science major. Attendees will be able to discuss assignment options for non-scientists and employ new tactics for expanding learning. Attendees will be able to outline coursework options using assignment examples provided and relate assignments to student feedback.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering background and ideas on how to teach complicated forensic science topics to non-science majors. Examples of coursework and student evaluations of the assignments are provided to allow educators to expand knowledge and assist in assessing projects to enhance student learning and application of knowledge.

Educating undergraduate students in a social science major on forensic science topics is especially challenging, as many have not taken a science course since high school. Teaching basic science is required for understanding of forensic science techniques and processes, which consumes valuable class time and can lead to student frustration. Complex forensic science topics can be successfully broken down using case studies and by showing the intersection of forensic science with social science-related criminal justice topics. The high-impact practice of incorporating collaborative assignments assists in increasing student involvement and learning to work in a team. The use of large-scale faux crime scenes incorporates collaborative assignments and gives students groundwork of basic science relating to different types of evidence. The students are responsible for recognizing evidence, photographing, collecting, processing, and recognizing the proper type of scientific forensic testing to request—showing application of the material. The crime scene also incorporates interviewing witnesses at the scene to employ knowledge and skills gained during the foundation portion of the course. Cold cases from local agencies are presented by detectives, and students are provided with the case files to review and “work” in a tabletop format. Using foundational knowledge gained from the beginning of the class, student groups review the evidence, assess the evidence and information available, and complete assignments based on other work or theories that can be followed. Student evaluations of forensic science-based activities in social science coursework show support of the assignments in analyzing cases, employing the basic science concepts learned, and interpreting evidence found during the faux crime scenes.

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**Forensic, Education, Non-Scientists**



### E116 The Educational Outreach Program at the Defense Forensic Science Center (DFSC)

*Rachel L. Creager, Defense Forensic Science Center, Forest Park, GA; Thomas A. Meyer, MS, Atlanta, GA 30311; Henry P. Maynard III, MSFS\*, Stockbridge, GA 30281*

**Learning Overview:** After attending this presentation, attendees will understand the DFSC's Educational Outreach Program (EOP) and the opportunities available to participate in cutting-edge research at DFSC.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing future forensic scientists and their academic mentors about available research options at DFSC that can advance their careers while furthering forensic science capabilities.

The Office of the Chief Scientist at DFSC performs one of the essential elements of the organization's mission by providing forensic research capabilities that support the Department of Defense (DoD). It is the mission of the Office of the Chief Scientist to increase the Army's readiness by executing an innovative Research, Development, Test, and Evaluation (RDT&E) program that strengthens current and develops future forensic science capabilities. For that reason, the DFSC EOP was established in 2011 to provide qualified university students an opportunity to conduct meaningful short-term research in a world-class forensic laboratory. The EOP introduces students to careers in the DoD, while building partnerships between DFSC and academia. Students receive a wealth of experience in experimental design, test and evaluation efforts, validations, and analysis of complex data. The Office of the Chief Scientist participates in various programs that allow students to work at DFSC, including the DoD Science, Mathematics, and Research for Transformation (SMART) program and the Army Educational Outreach Program College Qualified Leaders (referred to throughout as Research Associate) program. This presentation will benefit prospective students by highlighting these career-developing opportunities (a brief summary of each is provided below).

The Office of the Chief Scientist coordinates DFSC's participation in the SMART program. The goal of the SMART program is to increase the number of civilian scientists and engineers that are employed by the DoD laboratories. To do this, students at any level within their academic career (Bachelor's, Master's, or PhD) can apply to the SMART program to receive a full academic scholarship and future employment at a DoD laboratory upon degree completion.

Through the Research Associate program, undergraduate students come to DFSC to work on research projects for a summer, a semester, or a year. While in the Research Associate program, students receive a generous stipend. Our Research Associate program differs from traditional internship programs in that the students participate directly on a research project under the guidance of a DFSC research scientist. The Research Associate program allows students to gain valuable knowledge, skills, and abilities in experimental design, testing, and analysis from dedicated forensic mentors.

Since 2011, DFSC has had 169 EOP participants from 65 universities provide more than 75,000 hours on more than 100 DFSC-led research projects. The DFSC's Educational Outreach Program has been growing annually and these numbers will continue to rise. Come learn more about the opportunities at DFSC and join the research team!

*The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the DoD. Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, DFSC, U.S. Army Criminal Investigation Command, OPMG, DA, or DoD.*

#### **Educational Outreach, Internship, Research**





### E117 Improving Forensic Science Information Seeking

Laura Sare\*, Texas A&M University, College Station, TX 77843; Sarah Bankston, MS\*, Medical Sciences Library, College Station, TX 77843; Jeffery K. Tomberlin, PhD\*, Department of Entomology, College Station, TX 77843-2475; Samantha J. Sawyer, BS\*, Department of Entomology, Texas A&M University, College Station, TX 77842

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**Learning Overview:** The goal of this presentation is to describe the findings from a survey of forensic science professionals from a variety of disciplines on how they search for information related to their occupation. The researchers aim to discover how libraries can serve as gateways to provide assistance when seeking information, such as scholarly materials.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting strategies to potentially enhance the ability to locate scholarly materials applicable to their profession. Such skills could lead to efficient skills for also assessing these materials.

In order to provide the forensic science community with effective and useful educational materials, this survey was the first stage of an investigation on forensic science professionals' information search strategies. The researchers wanted to uncover how forensic scientists think about finding information in their specific fields, tease out strengths that could be shared throughout the forensic science community as a whole, and identify knowledge gaps in search strategies that could be improved upon and brought to the community through learning modules.

A survey created in Qualtrics®, comprised of 13 questions, was sent out on August 19, 2017, via the American Academy of Forensic Sciences (AAFS) listserv. The survey was open for responses until November 8, 2017. There was a total of 547 responses, and the dataset consists of the 333 completed surveys. Multiple choice response analysis was provided by Qualtrics®, while open response questions were coded using central themes for each question independently and analyzed.

While most forensic science professionals surveyed do not search for research material daily, many need to find information on a monthly or weekly basis. For those positions and disciplines that need to conduct research, a majority said they often could not access articles they located and would like to use due to paywalls. Those that did not have problems accessing articles were usually affiliated with a university.

Results of the survey indicate some forensic science professionals are in need of training for effectively locating scholarly research material. Three themes developed from this research. The first is that libraries and researchers have different definitions for information seeking terminology that need to be bridged. The second theme was a need for guidance on where to find quality materials and how to find open access (freely available research) materials for those who do not subscribe to journals. The last theme that emerged was the need for direction on how to create an effective search strategy using keywords and Boolean operators.

This project will determine how forensic scientists locate and evaluate information to allow the researchers to develop educational materials aimed at improving these skills and their efficiency with locating quality literature. Attendees will learn strategies potentially enhancing their ability to locate scholarly materials applicable to their profession.

*This research was funded by National Institutes of Justice grant 2016-R2-CX-0054. Opinions, points of view expressed in this research, and products discussed represent a consensus of the authors and do not necessarily represent the official position, policies, or endorsement of the United States Department of Justice, Office of Justice Programs, or the National Institute of Justice.*

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### Information Literacy, Science Continuing Education, Libraries



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### E118 A Journal of Our Own: *The Journal of Forensic Science Education*—The Official Publication of the Council of Forensic Science Educators

Lawrence Quarino, PhD\*, Cedar Crest College, Allentown, PA 18104; Kelly M. Elkins, PhD, Towson University, Towson, MD 21252; Adrienne L. Brundage, PhD, Bryan, TX 77807

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**Learning Overview:** After attending this presentation, attendees will be aware of a newly developed open-access journal titled *The Journal of Forensic Science Education* that is intended to be a resource to help forensic science educators: (1) develop pedagogy and curriculum appropriate to the forensic science discipline, (2) promote scholarship in forensic science education, and (3) serve as a communication resource between forensic science educators.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how *The Journal of Forensic Science Education* can further the goal of quality forensic science education by serving as a vital resource for educators that can help develop scientific competency in students at multiple levels.

An assessment of the forensic sciences, published in 1999 by the National Institute of Justice, described the educational and training needs of the forensic science community as “immense.”<sup>1</sup> This immense need has developed over the past three decades by the unprecedented demand of the criminal justice system for crime laboratory services due in large part to the development of technologies that can provide scientific information that previously could not be obtained. As a result of this demand, local, state, and federal government resources have been allocated to expand laboratory services, although the availability of individuals with the requisite skills and education to fill new positions is severely limited. To try and meet this need, undergraduate and graduate forensic science programs have developed at an unparalleled rate over the past two decades in the United States and abroad. An examination of the American Academy of Forensic Sciences website reveals more than 120 forensic science programs leading to a bachelor’s or master’s degree. This number is probably the proverbial “tip of the iceberg,” particularly considering that few international programs are listed. Considering that the vast majority of these programs did not exist 20 years ago is cause for optimism about the future of forensic science. However, at the same time, this rapid growth is cause for concern about the quality of many of these programs. In addition, the forensic science educational landscape is even bigger considering the number of high school science teachers who now teach forensic science courses, usually as popular science electives.

The Council of Forensic Science Educators (COFSE) was created more than 20 years ago with this goal in mind. COFSE objectives include the exchange of ideas and discussion of problems of common interest between forensic science educators and for educators to work collectively to upgrade the quality of forensic science education. COFSE is composed of individuals involved in forensic science education from the high school through graduate level. It has helped give rise to the “forensic science educator,” a concept distinct from educators of other scientific disciplines. COFSE is also the parent organization of Delta Delta Epsilon, an international honor society designed to recognize high-achieving forensic science students at the undergraduate and graduate level, furthering the idea of forensic science as more than simply an applied scientific discipline. At the annual meeting of the American Academy of Forensic Sciences in 2018, COFSE presented a full-day workshop on active learning in forensic science.

It makes sense, then, for COFSE to take the next step and establish an official publication. *The Journal of Forensic Science Education* will commence in 2019 and will be an international, peer-reviewed journal devoted to improving forensic science education pedagogy, laboratory and classroom activities, and promoting the scholarship of forensic science education. In many ways, it will be modeled after the *Journal of Chemical Education* offered through the American Chemical Society that has been in publication since 1924. Currently, the planning committee for the *Journal* is creating an editorial board and an on-line platform. The *Journal* will be open access and housed through the Texas Digital Library. Articles will be indexed through Google® Scholar and other databases. The *Journal* will welcome editorials, commentaries, letters, pedagogical articles, laboratory experiments, demonstrations, technology reviews, and books reviews. The *Journal* will serve to enhance communication among forensic science educators from high school to university and people interested in the teaching and learning of forensic science from forensic laboratories and government.

#### Reference(s):

<sup>1</sup> National Institute of Justice. *Forensic Sciences: A Review of Status and Needs*. 1999.

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#### Forensic Science Education, Journal, COFSE



### **E119 Is the Decentralization of Forensic Capabilities Providing an Opportunity for Forensic Science to Reach the End of the Crossroads?**

*Claude Roux, PhD\**, University of Technology Sydney, Broadway, NSW 2007, AUSTRALIA; *Eoghan Casey, PhD*, University of Lausanne, Lausanne, Vaud, SWITZERLAND; *Frank Crispino, PhD*, Université du Québec à Trois-Rivières, Trois-Rivières, PQ G9A 5H7, CANADA; *Olivier Delémont, PhD*, School of Criminal Justice, University of Lausanne, Lausanne, Vaud CH-1015, SWITZERLAND; *Olivier Ribaux, PhD*, Ecole Des Sciences Criminelles, Lausanne 1015, SWITZERLAND

**Learning Overview:** The goal of this presentation is to provide an outlook of forensic science and its future by considering the rapid and ongoing technology changes in modern society, especially regarding the decentralization of forensic capabilities and digital traces.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing guiding principles to be better equipped to face current and future challenges in forensic science. This presentation will encourage managers, educators, researchers, and policymakers to look beyond the usefulness of forensic results for solving individual investigations and to realize the value of combined forensic knowledge and intelligence for developing broader strategies to deal with crime in a globalized, digitalized society.

Forensic science has been at the crossroads, not to say in crisis, for more than a decade. The robustness of the scientific foundations of essentially all of the forensic science disciplines is being questioned on a regular basis. Further, the usefulness of forensic science continues to be questioned by evaluative studies focusing on the judicial contribution. In this debate, it is apparent that very few discussions considered the very nature of forensic science, overlooking essential contributions to investigation and security, beyond the single production of evidence in court. Further, some rapid changes in society and organizations are bringing additional challenges for forensic science laboratories. One such critical change is the expanding decentralization of forensic capabilities, particularly for digital traces, which exposes and catalyzes a rupture with the traditional central forensic science laboratory model. In this presentation, it is argued that this situation generates not only challenges but also opportunities for forensic science to move forward.

To assist this unavoidable decentralization, it is recommended that laboratories undertake digital transformations to capitalize on the decentralization movement, develop a more comprehensive understanding of crime and security-relevant problems, and play a more central role in problem solving collaboratively with law enforcement organizations and other stakeholders. A model for bilateral transfer of information and knowledge is proposed to magnify the impact of forensic science laboratories on abating crime, strengthening security, and reinforcing the criminal justice system. To accomplish digital transformations, laboratories require personnel with different expertise, including investigative reasoning, knowledge codification, data analytics, and forensic intelligence.

A policy window is open. Forensic science laboratories have the potential to achieve the broader legitimacy that is required for redefining their position through transformed processes. However, this requires some courage and conviction from the forensic science community and reciprocal commitment, including the creation of processes within law enforcement organizations and government that ensure the new knowledge generated by laboratories will be utilized effectively.

At that stage, one may have reached the end of the crossroads.

#### **Forensic Intelligence, Problem-Oriented Policing, Knowledge Management**



## E120 Perceptions and Estimates of Error Rates in Forensic Science: A Survey of Forensic Analysts

Daniel C. Murrie, PhD\*, *Institute of Law, Psychiatry, and Public Policy, Charlottesville, VA 22908*; Brett O. Gardner, PhD, *University of Virginia, Charlottesville, VA*; Sharon Kelley, PhD, *Institute of Law, Psychiatry, and Public Policy, Charlottesville, VA 22903*; Kellyn Blaisdell, BA, *University of Virginia, Charlottesville, VA 22902*

**Learning Overview:** The goal of this presentation is to educate attendees about commonly held beliefs of forensic analysts across multiple disciplines regarding the prevalence and acceptability of different types of errors (i.e., false positive and false negative errors) in their field.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing results of the first study to empirically assess forensic analysts' opinions and attitudes regarding the prevalence and acceptability of errors within their discipline, as well as to show common misperceptions relating to error rates among forensic analysts.

Historically, many forensic scientists have adamantly denied the presence of error in their field.<sup>1,2</sup> Critical reviews have noted that such statements are not scientifically defensible and have called for research to appropriately quantify error in all valid forensic science disciplines.<sup>3,4</sup> However, because such research is relatively recent and controversial, error rates in many forensic science disciplines remain unknown and even the best estimates are unreliable.<sup>3,5</sup>

The present study sought to evaluate how forensic analysts think about error in their disciplines and how demographic characteristics might influence such perceptions. In total, 183 practicing forensic analysts from wide-ranging disciplines completed a three-part survey at the outset of five training programs in the United States. For the current study, results are presented from the first two parts of the survey addressing: (1) attitudes regarding the acceptability of error types, and (2) estimates of error rates within forensic science.

Before asking questions about perceptions or estimates, participants were educated on false positive and false negative errors. Analysts then indicated how they "weigh and prioritize the risk of each type of error" on an 11-point scale ranging from 0=*I minimize the risk of false positives*, to 10=*I minimize the risk of false negatives*. Participants also completed similar questions indicating how they believed their laboratory, and discipline as a whole, weigh and prioritize the risk of each error type. In part two of the survey, participants estimated the false positive and false negative error rates in their discipline using a scale of 14 possible error rates ranging from "approximately 1 time in 2," to "such an error is impossible." After estimating error rates, participants either identified a specific source for their estimates or indicated that they did not know of any source documenting known error rates in their discipline.

Analysts generally perceived false positive errors to be less frequent than false negatives, although both error types were considered to be infrequent. Across disciplines, 38% of analysts indicated that the rate of *false positive errors* was equal to or less than one in one billion, with 10% stating that such errors were not possible. Likewise, 22% of analysts indicated that the rate of *false negative errors* was equal to or less than one in one billion, with 6% stating that such errors were not possible. Notably though, estimated error rates differed according to discipline, with forensic biology analysts typically estimating that errors were less common than did pattern evidence analysts. When asked to identify a source for their estimated error rates, the vast majority of analysts (78.7%) did not provide a source.

In general, analysts reported that they, their workplace, and their discipline prefer to minimize the risk of false positives and thus tolerate a greater risk of false negatives. Crime scene investigation analysts were the lone exception in that they held a more balanced view regarding minimizing false positive and negative errors. Additional detailed results and moderating variables (e.g., work experience) will be addressed at the conference.

In conclusion, this presentation will discuss how forensic analysts view error rates in their field and discuss implications for future practice and research. For example, although most forensic science disciplines do not have established error rates, the fact that more than one in five analysts estimated the risk of each error type to be impossible or very low (i.e., less than one in one billion) is concerning in light of: (1) the inevitability of errors, and (2) human vulnerability to cognitive bias.<sup>3</sup> Results highlight the need for further work investigating and disseminating error rates as a step toward informing policy and practice.

### Reference(s):

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5. Koehler, J.J. Intuitive Error Rate Estimates for the Forensic Sciences. *Jurimetrics Journal* 57, (2017): 153-168.

### Error Rates, Bias, Forensic Analysts



**E121 Response of Soil Microbial Communities to Mammal Decomposition During Above-Ground and Subsurface Decomposition: A Comparison**

*Chawki Bisker, MSc\*, Teesside University, Middlesbrough, North Yorkshire TS1 4PS, UNITED KINGDOM*

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**THIS ABSTRACT WAS NOT PRESENTED.**



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### E122 Forensic Archaeology and Search Strategies for Clandestine Burials: Geographic Profiling in Reverse and “Winthroping”

*Sharon K. Moses, PhD\*, Northern Arizona University, Flagstaff, AZ 86011-5200*

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**Learning Overview:** After attending this presentation, attendees will better understand how geographic profiling and “winthroping” may be useful tools for clandestine burial searches. This presentation will familiarize attendees with the basic principles of geographic profiling commonly used by law enforcement to locate an offender’s likely home base according to mapped patterns of their crime scenes. However, searches for clandestine burials need to be based upon geographic profiling patterns in reverse: the offenders’ home base (known or suspected), the victims’ home base, other related crime scenes as applicable, and the offenders’ habits to determine likely areas for a clandestine burial. Attendees will also be introduced to the concept of “winthroping,” which was originally developed by the military for the purpose of locating hidden munitions caches. Most clandestine burial searches are based upon tips, tracking offenders’ activities close to the disappearance date of a suspected victim, and general speculation combined with a hit-or-miss approach of volunteer groups who typically canvass areas around a victim’s last known whereabouts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing involvement of forensic archaeologists’ skills in conjunction with adapted geographic profiling strategies that can aid in identifying possible burial locations. Forensic archaeologists have been taught as part of their academic training to recognize and understand spatial dynamics of built and natural environments and human and animal movement across those landscapes. It cannot be overstated that these skills can offer important insights that an untrained eye may miss in an outdoor crime scene. This presentation promotes the involvement of forensic archaeologists to participate before the actual excavation of a clandestine burial and encourages law enforcement to utilize their skills in the search phase. Many times, a homicide is suspected but a body has not been found. Locating a body is problematic without a confession, and oftentimes cases are severely disadvantaged when the victim is not found.

This presentation will demonstrate that geographic profiling, originally conceived of as a means to locate offenders, is a tool that may also be adapted to focus on patterns and choices to find burials. This presentation will explore how an offender’s environment and relationship (or lack thereof) to a victim also influences choices for clandestine burial sites.

There is unique value in combining forensic archaeological skills with understanding multivariate landscapes in criminal activities, human connection to those landscapes, and consideration of unconscious and habitual behavior patterns toward identifying likely burial locations. Research on profiling in this context is currently very limited and this presentation is aimed at stimulating more exploration into, and development of, adapting and incorporating geographic profiles in conjunction with forensic archaeologists’ involvement in burial search strategies.

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#### Forensic Archaeology, Geographic Profiling, Winthroping



### **E123 Interagency Cooperation in the Recovery of Human Cremains and Human Remains**

*Rebecca Wood, MFS\*, Washington, DC 20024; Francisco J. Diaz, MD\*, Washington DC Office of the Medical Examiner, Washington, DC 20024*

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**Learning Overview:** After attending this presentation, attendees will understand what encompasses an incident action plan, how decedents are most commonly identified at the Washington, DC, Office of the Chief Medical Examiner (OCME), and the challenges faced when a funeral home is closed indefinitely.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by shedding light on a seldom-discussed topic: the closing of a funeral home and the role of the medical examiner therein. This presentation will also provide an overview of the way the DC OCME notified and interacted with next of kin as well as assisted with the reunification of families.

The Washington, DC, Office of the Chief Medical Examiner partnered with the DC Office of the Attorney General (OAG) and the United States Marshals Service in a small-scale operation involving the recovery of more than 100 cremains and several non-cremated human remains from a funeral home that was closing in Washington, DC. The operation was conducted over the course of two days, one day in November and one day in December of the 2017 calendar year. There was intensive preparation prior to the recovery, and this presentation will highlight the preparation, logistics, and field cataloging process.

This presentation will include an overview of the method the DC OCME used to identify cremains and the limitations this process exposed. This presentation will highlight challenges faced in an effort to assist other medical examiner/coroner offices who may encounter similar situations. Attendees will be provided with the methodology behind creating a field cataloging process, as well as an overview of the comprehensive Microsoft® Excel® spreadsheet created by the DC OCME to properly document, triage, and analyze the cremains and non-cremated human remains recovered during this small-scale operation. Additionally, a time-line will be provided to further assist in visualizing the long-standing effects such an operation can have on a medical examiner/coroner office, the other agencies involved, and the community as a whole (to include the next of kin).

This presentation will also impact the forensic science community by discussing interagency cooperation among organizations within three regions: the District of Columbia, the Commonwealth of Virginia, and the state of Maryland. Finally, this presentation will impact the forensic science community by encouraging other agencies to become aware of the possibility that they too may be called upon to assist in such a recovery mission.

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#### **Interagency Cooperation, Human Cremains, Family Reunification**



### E124 The Identification of Necrophagous Insect Species by Ambient Ionization Mass Spectrometry and Chemometrics for Postmortem Interval Estimations

Rabi A. Musah, PhD\*, State University of New York at Albany, Albany, NY 12222; Samira Beyramysoltan, PhD, State University of New York at Albany, Chemistry Department, Albany, NY 12222; Justine E. Giffen, BS, State University of New York at Albany, Albany, NY 12222; Jennifer Rosati, PhD, John Jay College of Criminal Justice, New York, NY 10019

**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the principles of ambient ionization mass spectrometry and its applications to forensic entomology. Additionally, attendees will learn about artificial neural networks and Self-Organizing Maps (SOMs) and how they can be generated from mass spectral data and utilized to determine the species identity of sample unknowns.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the utility of ambient ionization mass spectrometry techniques in forensic entomology. The ability to rapidly analyze and identify necrophagous insects discovered on a corpse, or in animal or elder care abuse cases, will reduce some of the challenges and shortcomings of traditional species identification methods and enable more rapid analysis and species identification.

Conventional methods of species identification of necrophagous insects for determination of the postmortem interval in medicolegal investigations are often time- and resource-consuming. Furthermore, the results can easily be influenced by a number of factors, including the manner in which death occurred, the environment and temperature in the area of the body, and the climate and time of year. Presented here is an alternative mass spectrometric and chemometric approach to species identification of blow flies at each of their life stages (eggs, larvae, pupae, and adults). Application of this approach has revealed: (1) information regarding the biomarkers responsible for the differentiation between species at each life stage, (2) that even closely related species can be differentiated, and (3) that SOMs derived from mass spectral data provide a highly accurate means of identifying insect species.

Egg, larva, pupa, and adult stage samples of blow flies belonging to six species within the order Diptera were collected and suspended in 70% aqueous ethanol storage solutions, which is the conventional practice for storage of entomological evidence discovered on or near a corpse. These solutions were then rapidly analyzed by Direct Analysis in Real Time-High-Resolution Mass Spectrometry (DART<sup>®</sup>-HRMS) to determine the unique chemical fingerprint spectra. These analyses were performed in replicates of five. The mass spectra were then subjected to chemometric processing in the form of Kohonen SOMs. After initial preprocessing of the raw mass spectral data, minimum Redundancy Maximal Relevance (mRMR) and Sparse Discriminant Analysis (SDA) methods were applied in order to reduce the data to contain only the most significant variables required to create the models. The reduced data were then separated into training and validation sets in order to test the prediction abilities of the models. Mixtures of several species' ethanol suspensions at a given life stage were then created and analyzed to further test the models.

The results indicated that the DART<sup>®</sup>-HRMS spectral profiles of the blow flies analyzed were highly consistent between replicates but showed interspecies and life stage differences. These fingerprint profiles were then used as the basis for the creation of SOM models, which revealed the mass-to-charge ( $m/z$ ) values of the chemical components that were the most important for discriminating between and identifying different species at each life stage. The cross-validation results for the predictive ability of each life stage SOM were above 93%, and the external validation accuracy was 100% for all models. In the case of the mixture samples, preliminary results indicate 97% accuracy.

The DART<sup>®</sup>-HRMS chemical fingerprint profiles that were generated were found to be suitable for chemometric processing in order to accomplish species discrimination. DART<sup>®</sup>-HRMS is a rapid means to rapidly collect unique mass spectra for many different species and life stages of necrophagous insects. The SOM models that were built can be further expanded to include more species and families of insects and create a robust database to assist in the determination of insect species identity and by extension, postmortem interval.

#### DART<sup>®</sup>-HRMS, Entomology, Chemometrics





### E125 The Ability of Cotton, Polyester, and Wool to Retain Diatomaceous Evidence Following Submersion to Study the Postmortem Submersion Interval (PMSI)

Alexandra G. Rizzuto, BS, Keystone College, La Plume, PA 18440; Jillian Conte, PhD\*, Keystone College, La Plume, PA 18440

**Learning Overview:** After attending this presentation, attendees will understand how common fabrics retain diatomaceous evidence and how this information can be useful in an investigation involving victim submersion in a body of water.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by advancing the knowledge of how diatomaceous materials are retained by different fabrics, assisting in the determination of crime scene location, transfer events, and, potentially, the PMSI.

Diatoms are unicellular, photosynthetic algae that are classified as *Chrysophyta* and Class *Bacillariophyceae*.<sup>1,2,3</sup> The microscopic nature of diatoms, which range in size from less than 5 to more than 500 microns, results in accumulations of 25-50 million cells in just one cubic inch of diatomite.<sup>1</sup> The relative abundance and small size of diatoms makes them ideal for forensic geoscience applications.<sup>4</sup> This area of forensics deals with comparison of environmental samples to establish or refute a common source.<sup>4</sup> The use of diatomaceous evidence in comparative trace analysis and relating it to postmortem events is currently an understudied area.

Historically, the forensic application of diatomaceous evidence has been limited to the diagnosis of drowning deaths.<sup>2</sup> The diatom test has been used for this purpose since 1942 and is based on the premise that diatoms do not occur naturally in the human body.<sup>3</sup> Diatoms enter the circulatory system and deposit themselves in organs, such as bone marrow and the brain.<sup>2</sup> Verma suggests that diatom analysis can be enhanced through analyses of submerged clothing or footwear in contact with the suspected drowning medium.<sup>3</sup>

The necessity of research into the forensic geoscience application of diatoms is further enhanced by the potential for diatoms to be utilized for the determination of PMSI.<sup>4</sup> The PMSI is the time interval between submersion and time of discovery of an item or victim.<sup>6</sup> Diatoms are initial colonizers of aquatic systems and they are dominant during algal succession.<sup>6</sup> This makes diatoms ideal organisms for PMSI determination. Zimmerman & Wallace were the first to develop a semi-quantitative approach to PMSI determination based on diatom diversity as a function of time.<sup>6</sup>

To date, only two studies have been conducted regarding the extraction of diatoms<sup>4,5</sup> from clothing, and both studies strictly used cotton.<sup>4,5</sup> Since clothing material is frequently recovered from crime scenes, it is important to understand how a variety of fabrics will retain trace materials. In forensic science, it is equally important to use optimized extraction techniques that provide a representative sample. Scott et al. developed a novel hydrogen peroxide extraction technique that yielded the highest number of diatoms and the most representative samples in comparison to the rinsing with water and rinsing with ethanol techniques.<sup>4</sup> Since the other two methods were not as representative of the reference samples, these should not be used in a forensic application where species composition is analyzed to determine source. To build on the current knowledge of how diatomaceous evidence is retained by fabrics, the following research question was proposed: Will the type of fabric affect the number of diatoms that are retained?

A 30% hydrogen peroxide extraction method was performed for three common fabric types (cotton, polyester, and wool) to retrieve embedded diatomaceous material. Resulting yields were examined using light microscopy and scanning electron microscopy. Slides were mounted with a mounting media suitable for the refractive index of diatoms. The research aimed to optimize the method for diatom extraction and compared the ability to yield diatomaceous evidence from cotton, polyester, and wool. The results for the optimized method were analyzed by one-way Analysis of Variance (ANOVA). An analysis of variance showed that the effect of type of fabric on the number of diatoms retained was significant,  $F(2,27)=32.067$ ,  $p=0.000$ . A Tukey post hoc test was conducted to determine which fabrics were different in retention. A relationship was found between cotton and polyester ( $p=0.206$ ). All other fabrics were significantly different ( $p<0.05$ ). This research furthers the knowledge of the forensic community by providing information about how different fabrics retain diatomaceous evidence.

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#### Postmortem Submersion Interval, Diatoms, Pathology



### **F1 A Jurisprudential Analysis of the DNA Exam and Its Use as Evidence in Decisions of Brazil's Superior Court of Justice**

*Maria Eduarda A. Amaral\*, Porto Alegre, Rio Grande do Sul 90619-900, BRAZIL; Nereu J. Giacomolli, Porto Alegre 90619-900, BRAZIL*

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**Learning Overview:** The goal of this presentation is to evaluate decisions of Brazil's Superior Court of Justice and to try to identify the importance given to the DNA exam as evidence in the decision.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a study about the scientific evidence and the DNA exam. This presentation will also initiate a discussion about the distance between the speech of different science areas and will examine the importance of collaboration and interdisciplinary communication.

A case law search was conducted on the website of the Superior Court of Justice on July 11, 2018, using the keywords "laudo pericial E DNA E penal" (the search was made in Portuguese).

Ten judgments were analyzed. The totality of the cases involved crimes of rape and/or indecent assault. In one case, the defense argued that it would be necessary to perform a DNA exam to prove the convicted person's ascendancy toward the victim. In this case, the magistrate understood that it would not be necessary to perform DNA, since the victim was adopted, and an ancestry also assumed in such cases. In another decision, the defense alleged that the collection of DNA occurred in an illegal manner and that the evidence would be unlawful. However, the magistrate understood that the DNA test was not the only element of evidence and that the collection was not illegal. In another decision, the defense requested the annulment of the judgment due to the lack of DNA evidence, and the magistrate understood that there was sufficient evidence and a DNA exam wasn't necessary. Furthermore, prior to the updating of the Brazilian Criminal Procedure Code conducted in 2008, it was necessary that the report be carried out by two official experts. At that time, appeals went to the Superior Court of Justice asking the validity of the expert report because it was not conducted by two official experts, and their qualifications were in question. In most of the decisions, report irregularities were not recognized or corrected. Thus, the reports were not used.

There is no consensus on the subject. The DNA exam is sufficient and robust evidence to support the decision for some judges. Others believe that more evidence is needed. Regarding the defense, it is noticeable that the DNA exam is highly defended or neglected depending on the importance that it may have for the defense. There is not a more rigorous assessment of the scientific method used to do the DNA exam.

The DNA exam was designed to be used in the medical science area and, over time, has been incorporated into the forensic sciences. However, legal writers frequently discuss about the methodology used in the DNA exam, including the collection process and handling, without deep knowledge about it. They question the accused's fundamental assurances and how the DNA exam could affect it. Thus, a discussion between both areas is extremely important so judges can understand what the DNA exam can be used for and what the limitations are regarding application to the law. In addition, forensic scientists will be able to realize that, although its technique is approximately 99.9% reliable, it will not always be used.

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**DNA Exam, Forensic Science, Penal Process Evidence**



### F2 *Iter Criminis* and *Mens Rea*: A Methodology to Understand an Offender's True Intention Based on a Revision of the Concept of Guilt in Italian Criminal Law

Vincenzo Lusa, JD\*, Rome 00151, ITALY; Annarita Franza, PhD\*, Department of Experimental & Clinical, Florence, ITALY

**Learning Overview:** After attending this presentation, attendees will understand the importance the Italian legal process provides to the criminal path (*iter criminis*), seeing as it is fundamental to undertaking an evaluation of an offender's true *mens rea* or the degree of culpability or insanity.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by acquainting attendees with the advantages of using a criminal path legal model, which can split the criminal's behavior and thus define his actual intent and mental state.

A criminal path study can also ascertain whether the offender suffers from insanity, which may prove useful during the trial from a psychiatric and psychological point of view, with definite advantages for the defense, the accused, and the jury. In Italy, the criminal path is associated with an attempted crime, as set out in article 56 of the Italian criminal code. It is used to identify the moment in which the illegal activity of an agent becomes punishable. Article 56 states that: "whosoever carries out acts clearly intended to commit a crime, is responsible for an attempted crime, if the action is not completed or the event does not occur." In Italy, Article 56 is occasionally placed with such specific incriminatory laws as attempted murder (art. 56, 575 Italian Criminal Code).

The criminal path consists of the following parameters: (1) ideation, in which the offender sketches out the initial purpose on which the crime is based. In fact, the agent outlines in his psyche the criminal idea he wants to commit, which is then manifested by its conclusion; (2) preparation, where the agent arranges everything needed to execute the crime; (3) execution, where the agent carries out the conduct required for the crime to occur; and (4) perpetration, when the crime is committed. To be precise, the crime has been completed with all the elements defined by criminal law.

Intention must not be confused with the agent's volition, since it appears because of criminal intention. In fact, the latter constitutes the guilty party's true evil animus (cited in art. 108 of the Italian Criminal Code). Volition is seen as the manifestation of criminal intent (the latter is *mens rea* in the United States and *dolo* (malice) in Italy), which is completed with the offender's action. Volition is maintained until the crime has been concluded; even mental pathologies leading to incapacity to understand and to want can be inferred once a reliable diagnosis of the criminal path has been undertaken (as they are attached to the latter). In this regard, forensic neuroscience should also be considered. Regarding conscience and volition of action (*suitas*), what about taking criminal responsibility into account with criminal psychiatry? If this is not done, liability is correlated to the ability to be in full possession of one's faculties. Article 85 of the Italian Criminal Code states that no one can be punished for an act seen by law as a crime "if, at the moment of the act, [that person] was not responsible" and that "those who are in full possession of their faculties are responsible." It can be said that consciousness and volition may be considered as separate from a capacity to understand and to want, since mere consciousness and volition do not include the ability to comprehend the negative social value of actions that the offender performs and which, perhaps, in a moment of sanity, would never have been carried out (art.85, Italian Criminal Code). Finally, a full legal and scientific model will be suggested that can be used to evaluate an individual's actual *mens rea* through the criminal path.

#### *Mens Rea, Iter Criminis, Liability*

### F3 Judicial Aspects of Suicide Inducement in the Turkish Judicial System and a Review of the Erol Cetin Case

*Oktay Cavus, MSc\*, Üsküdar University, Istanbul 34430, TURKEY; Hatice Yılmaz, BSc\*, Üsküdar University, Istanbul 34662, TURKEY; Sevil Atasoy, PhD, Uskudar Universitesi Adli Bilimler Enstitüsü, Istanbul, TURKEY; Tugba Ünsal, PhD, Üsküdar University Department of Forensic Science, Istanbul 34662, TURKEY*

**Learning Overview:** After attending this presentation, attendees will have a tool for comparative analysis of judicial aspects of suicide inducement and its application in Turkey.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an insight about the judicial aspects, code of conduct, and case report of suicide inducement in the Turkish judicial system.

Suicide has been interpreted in different forms throughout history. In general, it is considered as a violation of social order and rules and as a threat thereto. One who commits suicide is considered to be an offender both from religious and social points of view and thus, they are ostracized. All suicidal attempts are associated with multiple reasons, and social and individual influences are thought to play a role in such attempts. Since suicide, described as an individual's will to end his/her own life, is no longer considered as a crime, any involvement in a suicide attempt of such an individual is also excluded from punishment. To close this gap, suicide inducement is regulated as a crime in its own right. As a matter of fact, Article 84 of the Turkish Penal Code, Article 115 of the Swiss Penal Code, Article 78 of the Austrian Penal Code, and Articles 223-13 and 223-14 of the French Penal Code stipulate sanctions thereof. However, it is interesting that in contrast to other countries, in German Criminal Law, suicide inducement is not regulated as a crime and therefore, just like suicide itself, suicide inducement or providing the means therefor are also not punished. In Germany, the issue is handled within the framework of the causal agency. No one can be punished due to attempting to commit suicide. Because in such a case, a suicidal person will be put under pressure through punishment norms, and thus will be induced to commit suicide. If it is opted to punish an individual with suicidal tendencies, this will incite a person to commit suicide and achieve a result thereof. As it is not possible to recognize such sanction, such an approach will not be welcome by the function of Criminal Law. Suicide inducement is regulated by Article 84 of the Turkish Penal Code; it can be committed as an immoral offense.

If an individual commits suicide, the suicide inducer will be punished more severely. For a milder form of suicide inducement, the punishment is imprisonment for two to five years if a suicide attempt does not result in death, whereas if it results in death, it is stipulated that imprisonment will be four years to ten years. The instigator (involved in stimulation intention and decision-making by a suicidal person), inducer (involved in decision-making by a suicidal person), or the ones who intensify such a decision or help the suicide be committed will be punished. Suicide inducement is a crime in the nature of a willful act, and the offender may have committed a concrete part of such a crime by being involved in acts set forth in Article 84/1 of Turkish Penal Code. In the case that a causality relation cannot be established between suicide and suicide inducement, it will not be possible to talk about such a crime as suicide inducement. Suicide inducement is committed with a general wrongful intention. The causality relationship should be established between suicide inducement, a suicide attempt, and a suicide, and, secondarily, the inducer of suicide should have a wrongful intention thereof. For determining qualification of a suicide inducement act, the person influenced by such an act should have at least attempted to commit suicide. In such a case, suicide inducement is not eligible for an attempt, for such a crime to be called, the existence of attempter of suicide influenced by the inducer of suicide is a must, and such an outline is set forth in Article 84/1 of Turkish Penal Code, which regulates milder forms of suicide inducement. If an offender induces more than one person to commit suicide through the instrument of the same concrete act, the degree of punishment will be increased since the same crime is committed against multiple individuals.

Two offenders, who induced Erol Çetin to jump from the Bosphorus Bridge in İstanbul, Turkey, on August 3, 2016, are currently being tried in court and are expected to be convicted.

#### **Suicide, Suicide Inducement, Sanctions of Suicide Inducement**



### F4 The Theory of Criminal Relativity: Using Genealogy Databases to Solve Crimes

*Donald E. Shelton, JD, PhD\**, University of Michigan-Dearborn, Dearborn, MI 48128-2406; *Frederick R. Bieber, PhD\**, Brigham & Women's Hospital, Boston, MA 02115

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**Learning Overview:** After attending this presentation, attendees will have a deeper understanding of how genealogy DNA databases are being used to solve crimes and the technical, legal, and ethical issues involved.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by giving attendees the ability to understand how genealogy DNA databases are being used to solve crimes and the technical, legal, and ethical issues involved.

In 2018, using DNA-based genealogical research, police were able to arrest a former police officer who allegedly committed at least 12 murders, more than 50 rapes, and over 100 burglaries in California between 1974 and 1986. In numerous cases after the arrest of the suspect in the “Golden State Killer,” law enforcement investigators are now using genealogical databases in a different and much more effective way than previously available methods have allowed. The use of DNA Single Nucleotide Polymorphism (SNP) profiling has significantly changed the face of genealogical searching and those changes now offer new investigative methods of crime solving. Initially, DNA typing methods revolutionized the criminal justice system and led to the development of the Combined DNA Index System (CODIS) database of persons who had contact with the criminal justice system (prisoners, convicts and, in some states, arrestees). Subsequently the ability to extract DNA from ever-more-minute crime scene samples amplified its use in criminal investigations. Short Tandem Repeat (STR) -based familial searching made it possible to search CODIS at the state level—to search not just for perfect matches to crime scene evidence, but also for very close matches to possibly identify close (first-degree) relatives of the donor of a crime scene specimen.

Now, new DNA-based public genealogy databases can be searched for even more distant relatives of whoever leaves crime scene DNA evidence, regardless of whether their profile is in CODIS. Several new open-access genealogy databases now enable law enforcement to search for scores of even distant relatives by comparing thousands of SNPs typed from crime scene DNA. Investigators can submit the raw data files from the crime scene DNA to one of the new genealogy databases to find possible relatives of the person who left the evidence. While the suspect may not be in CODIS and may not have submitted his or her DNA to the genealogy site, the DNA of any of his or her relatives who have innocently submitted their data for genealogy search purposes can lead police to a range of possible persons of interest.

The SNP comparison process will be explained and differentiated from other traditional DNA-based familial searches. The results of DNA-based genealogy searching still require careful data analysis and follow-up investigation by police to further narrow the range of possible persons of interest based on age, locale, and other factors associated with the crime. Once a person of interest has been identified, police then need to obtain a known DNA sample from that person. This has been done surreptitiously using discarded DNA without a warrant (e.g., reportedly from a discarded tissue in the “Golden State” investigation). After that DNA profile is related to the crime scene specimen, the police then have the basis for a warrant. The entire process may raise potential issues under the Fourth Amendment, particularly considering the recent *Carpenter* United States Supreme Court opinions, as well as online e-privacy laws. The social, ethical, and policy implications of genealogics will also be discussed.

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### Genealogy Databases, DNA, Crime and Genealogy



### F5 A Proposal for Legislative Oversight of Genealogical Websites

*Stephen B. Mercer, JD\*, RaquinMercer LLC, Rockville, MD 20852; Isabelle R. Raquin, LLM\*, RaquinMercer LLC, Rockville, MD 20852*

**Learning Overview:** After attending this presentation, attendees will understand the novel aspects of informational privacy for DNA and the importance of legislative regulation and oversight of DNA collection, retention, and distribution practices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a framework for evaluating DNA data banking practices and responding to ethical concerns with legislation that balances legitimate law enforcement and public health objectives with individual and family privacy interests.

DNA databanks are proliferating outside of the Combined DNA Index System (CODIS) and the regulatory framework that presently governs the collection, retention, and distribution of DNA samples and records. These shadow DNA databanks primarily exist at the local level and are maintained by local police agencies, by private companies that sell databank related services to police, and by private companies that sell direct-to-consumer test kits for genealogical and medical information.

Underregulated DNA databanks are a concern precisely for the reasons that explain their existence and proliferation: (1) police are able to avoid regulation and oversight of who they collect DNA from and how they retain or use DNA to investigate crime; (2) police can broaden the types of crime DNA databanks are used to investigate to more discretionary-based offenses (e.g., property crimes and other quality of life offenses); (3) police can perform familial searching in a state such as Maryland that prohibits the practice in its statewide databank; and (4) private companies with large databanks of DNA can have disproportionate influence over the course of medical research.

Although individual cases of law enforcement searching of genealogical websites have been widely reported, the overall utility of these underregulated public and private DNA databases is presently unknown because there are no uniform standards for the reporting of data about whose profiles have been placed in local or private DNA databanks, why or how DNA samples have been collected, and how DNA samples are retained, used, or further distributed. Without such reporting, the public and state lawmakers cannot know whether local or private DNA databanks are being misused or further linked to other public or private databanks. Moreover, any disparate impact or effect by race, ethnicity, or class will not be known.

Courts have largely taken a hands-off approach to regulating DNA databanks. Legislatures have recognized the need to regulate DNA collected and retained for use in CODIS, but only a few states have expressly addressed local databanks and no state has addressed the regulation of private DNA databanks maintained for genealogical or medical purposes but are also used to facilitate criminal investigations.

This lack of legislative oversight for local DNA databanks is troubling. Since the beginning of law enforcement, DNA databanking with the enactment of the federal CODIS law in 1994, the importance of legislative regulation, and oversight of DNA collection practices have been widely recognized. DNA databases “inevitably reflect the race, class, and geographic biases imbedded in police and judicial practices,” and as the former dean of the University of Maryland’s law school has noted, “[f]rom 1990 to 2004, Blacks were five times more likely than Whites to be incarcerated, and in 2000, Blacks and Latinos comprised 63% of incarcerated adults, even though together they represented 25% of the total population”—trends that are attributable to “racial profiling, discriminatory sentencing, and general racial bias in the criminal justice system ....”<sup>1,2</sup> Exactly because of the disproportionate representation of historically disfavored groups in the criminal justice system, they are also disproportionately represented in governmental DNA databanks.

On the other hand, it has been noted that private genealogical databases are skewed by race and class in the opposite direction. This has implications for the use of genealogical databanks to develop “personalized” medicine that target favored groups.

DNA is a powerful investigative tool to identify and apprehend criminal offenders, but its potential for abuse is real and must, therefore, be subjected to close legislative scrutiny and supervision. Statutory regulation of local police DNA collection and databank practices will clearly advance these ends. Model language is proposed for regulating local and genealogical DNA databanks.

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#### DNA, Databank, Genealogical



### F6 Results of a Survey on Probabilistic Genotyping From the Legal Community

Michael D. Coble, PhD\*, University of North Texas Health Science Center, Fort Worth, TX 76107

**Learning Overview:** After attending this presentation, attendees will better understand the current state of training and the issues surrounding the use of probabilistic genotyping from the perspective of the legal community.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science and legal communities by helping to: (1) determine how well the legal community is coping with the current change in DNA mixture interpretation using probabilistic genotyping software, and (2) show where and how training can help to improve the litigation of mixture interpretation and reporting using probabilistic genotyping software and likelihood ratios.

DNA mixtures of two or more individuals can be challenging to interpret for the forensic DNA scientist. Although guidance for autosomal Short Tandem Repeat (STR) interpretation of DNA mixtures from organizations such as the Scientific Working Group on DNA Methods (SWGDM) and the International Society for Forensic Genetics (ISFG) have been provided, the challenge of interpreting complex mixtures remains an issue for the forensic DNA community.<sup>1-3</sup>

Probabilistic genotyping, according to SWGDAM, “refers to the use of biological modeling, statistical theory, computer algorithms, and probability distributions to calculate likelihood ratios and/or infer genotypes for the DNA typing results of forensic samples (“forensic DNA typing results”).”<sup>4</sup> Over the past decade, there has been an “explosion” of software that uses a probabilistic approach to mixture interpretation. *All* of these programs use the likelihood ratio to convey the value of the evidence.

There are at least 45 laboratory systems in the United States that are currently using a probabilistic genotyping software system, with another 56 laboratory systems in some state of purchasing, validating, or implementing a program.<sup>5,6</sup> We are now at a tipping point for the use of probabilistic methods of mixture interpretation as the new “standard” for the forensic DNA community.

This presentation will provide the perspective of the legal community, through a survey conducted in late 2018, on the movement away from “binary” methods of interpretation for DNA mixtures to probabilistic methods of interpretation. Survey participants were asked about their access to training in probabilistic genotyping and likelihood ratios, how well they understand this change in interpretation and presentation of the results in court, and how well juries and judges have accepted these changes. The results will also provide advice from both prosecution and defense attorneys on issues involved with understanding and presenting this approach in court. Finally, a summary of training materials, literature, and other resources for the legal community will be presented.

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#### Probabilistic Genotyping, DNA Mixture Interpretation, Likelihood Ratio



### F7 The Strengths and Limitations of Transfer DNA Evidence Recovered From Objects Handled by Multiple Individuals

*Leann G. Rizer, BS\*, Ypsilanti, MI 48197; Jonah W.P. Stone, BS, Indianapolis, IN 46241; Krista E. Latham, PhD, University of Indianapolis, Indianapolis, IN 46227; Cynthia Cale, MS, Strand Diagnostics, Indianapolis, IN 46241; Gay L. Bush, PhD, Strand Diagnostics, Indianapolis, IN 46241*

**Learning Overview:** After attending this presentation, attendees will better understand the strengths and limitations of transfer DNA evidence in situations in which multiple people handled the same objects and in which multiple DNA transfer events occurred. The goal of this presentation is to demonstrate the difficulty of predicting the mode of DNA transfer and handling order from DNA evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding to a growing body of knowledge regarding transfer DNA and DNA evidence interpretation in medicolegal investigations.

Advances within the field of forensic science have allowed for an increase in the sensitivity of various technologies involved in the detection and typing of DNA evidence. As this sensitivity increases, so does the likelihood of detecting and amplifying DNA not associated with the criminal act. To better understand, explain, and interpret DNA recovered from evidentiary items, one must have a comprehensive understanding of the active and passive processes that lead to DNA deposition, as well as the numerous variables that influence the transfer of DNA between individuals and objects. Recently, there has been an increase in the number of transfer DNA samples submitted to forensic DNA laboratories for analysis. As a result, scientific studies regarding transfer DNA need to be conducted to better understand the strengths and limitations of transfer DNA evidence. Conducting systematic studies that test the limits of DNA transfer, understanding the factors that influence DNA transfer, and furthering the understanding of DNA transfer in various real-world scenarios will only act to bolster the interpretation of scientific evidence presented in criminal investigations.

This study expands upon transfer DNA research conducted by Goray and van Oorschot and explores the transfer of DNA in a simulated social setting.<sup>1</sup> Four participants handled pre-sterilized plastic objects (a jug and four cups). This served to simulate a social event in which multiple people pour beverages from the same jug in an open-air setting, as would be encountered in a restaurant or bar. The order and timing of handling were predetermined and controlled by the researchers to test for the various modes of DNA transfer (primary, secondary, and tertiary). The current study tests the following null hypotheses: (1) secondary DNA transfer will not occur and will not be detected on dominant hands, (2) tertiary DNA transfer will not occur and will not be detected on the cups, (3) a mixed DNA profile from all participants will not be found on the jug handle, and (4) the order of handling the objects cannot be discerned from the DNA data. The dominant hands of the participants as well as the cups and jug were swabbed at various times throughout the study to test for evidence of DNA transfer. The DNA was analyzed and interpreted following standard operating procedures.

DNA was detected in 92% of the samples collected in this study. However, only 50% of those profiles met casework requirements for interpretation utilizing the laboratory's current interpretation guidelines. Eighty percent of these samples were mixtures containing DNA from two or more contributors, and 60% had identifiable major and minor contributors. There was no correlation found between the DNA profiles obtained from the handled objects and the timing or length of contact the participants had with those objects. For the samples obtained from the participant's hands, the major profiles produced were consistent with the individual from which the swab was taken. Seventy-five percent of the profiles obtained contained extraneous DNA leading to complex mixtures.

These results illustrate the complex nature of interpreting DNA transfer that occurs in a social setting involving multiple individuals and multiple transfer events. In this study, the objects were pre-sterilized, the order of handlers and length of contact was recorded, and the handlers' profiles were known. Despite these controlled aspects, the order and timing of handling could still not be reconstructed from the DNA profile information. This study demonstrates the ease of DNA transfer between objects and individuals, illustrates the difficulty in predicting mode of DNA transfer based on the DNA typing results, and highlights the difficulty in interpreting the genetic data when multiple transfer events have occurred.

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#### DNA Transfer, DNA Evidence, DNA Mixtures





### F8 Indirect DNA Transfer: The Impact of Contact Length on Skin-to-Skin-to-Object DNA Transfer

Cynthia Cale, MS\*, Strand Diagnostics, Indianapolis, IN 46241; Krista E. Latham, PhD, University of Indianapolis, Indianapolis, IN 46227; Stephen P. Nawrocki, PhD, University of Indianapolis, Indianapolis, IN 46227-3697; Gay L. Bush, PhD, Strand Diagnostics, Indianapolis, IN 46241

**Learning Overview:** After this presentation, attendees will appreciate the complex nature of DNA transfer. The goals of this presentation are to: (1) evaluate the indirect transfer of DNA to an object when a person serves as the vector of transfer, (2) to investigate the length of contact between two individuals that might result in the indirect transfer of DNA, and (3) to assess the impact of indirect DNA transfer on determining how evidence relates to a crime scene.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that indirect DNA transfer to an object can occur through brief skin-to-skin contact and that it can have a significant impact on understanding evidence in relation to a crime scene.

The following null hypotheses were tested: (Hn1) a single DNA profile of the direct handler will be the only DNA identified on each knife, and (Hn2) transfer DNA from the non-handler will result in an inconclusive profile unsuitable for statistical analysis.

The analysis of trace amounts of DNA from items possibly handled by a suspect during the commission of a crime often plays a crucial role in criminal investigations. In some cases, DNA left on an object can be the only link to the perpetrator. The increased capability to detect minute traces of DNA from a perpetrator at a crime scene has been a continuous goal of the forensic community. The increase in sensitivity in most cases is beneficial. However, it can also lead to the detection of extraneous DNA not related to the forensic investigation, which can complicate the identification of a suspect and increase the uncertainty of its evidentiary value.<sup>1</sup>

Empirical research has not only demonstrated the primary transfer of DNA via direct contact with an object, but also the indirect transfer of DNA whereby an individual's DNA is transferred to an object or another individual via an intermediary. The indirect transfer of DNA as an explanation for the presence of trace DNA samples at a crime scene appears to be becoming more prevalent in forensic investigations and during subsequent court proceedings.

This scientific research used handshaking to simulate contact that could lead to the indirect transfer of DNA. Participants shook hands continuously for varying lengths of time: 10 ( $n=24$ ), 30 ( $n=24$ ), 60 ( $n=24$ ), and 120 ( $n=24$ ) seconds. Knives were handled immediately following handshaking and were sampled after two minutes of handling. The samples were analyzed and interpreted following standard operating procedures.<sup>2</sup>

Data was obtained from 92 of 96 samples. Interpretable DNA profiles were obtained from 48 samples. The DNA yields for samples that resulted in interpretable profiles ranged from 50pg to 5ng. In 69% of the interpretable profiles, single-source profiles or mixed DNA profiles with the major component matching the primary contributor were obtained. Contributor inversions, where the non-handler matched the major component of a mixed DNA profile were observed: five at 120 seconds, four at 30 seconds, and one at 10 seconds. Indistinguishable mixtures of both contributors were obtained from four samples, making it difficult to identify the direct knife handler. In the remaining 44 samples, the presence of non-handler DNA and/or extraneous DNA rendered the profiles inconclusive.

The results of this scientific research support the concept that the identification of a DNA profile on an object cannot be considered proof of direct contact. Likewise, the amount of DNA recovered and/or the quality of the DNA profile obtained from an object cannot be used to infer the mode of transfer.<sup>3</sup> In addition, these results demonstrate that under experimental conditions that maximize DNA transfer and recovery, indirect DNA transfer to an object can occur with as little as ten seconds and as much as two minutes of continuous skin-to-skin contact.

The presence of indirectly transferred DNA on an object can make the identification of the direct handler difficult. DNA transfer does not render DNA evidence unreliable but represents an additional level of complexity in the analysis and interpretation of DNA profiles. Forensic DNA analysts should provide the court with an unbiased list of all possible modes of DNA transfer. In addition, forensic DNA analysts need to proceed with caution when addressing activity-level questions and base their opinions on published peer-review data rather than giving anecdotal responses.<sup>1,4,5</sup>

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#### Transfer DNA, Indirect DNA Transfer, DNA Evidence Interpretation

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### F9 Investigating the Potential for Transfer DNA on Laboratory Gloves

*Caitlin C.M. Vogelsberg, MS\*, Michigan State University, East Lansing, MI 48824; Krista E. Latham, PhD, University of Indianapolis, Indianapolis, IN 46227; Cynthia Cale, MS, Strand Diagnostics, Indianapolis, IN 46241; Gay L. Bush, PhD, Strand Diagnostics, Indianapolis, IN 46241*

**Learning Overview:** After attending this presentation, attendees will understand the potential for transferring DNA to laboratory gloves and boxes during routine laboratory use. The goal is to identify technician-mediated contamination and prevent it through the development of appropriate quality control procedures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that while the transfer of DNA from technicians to routinely used laboratory items, such as gloves and glove boxes, is possible, current contamination prevention protocols in laboratories with restricted access may be sufficient in reducing this potential source of error.

DNA evidence is often a key factor in criminal cases and can have a profound impact on the outcome in the courtroom and the lives of the accused. Therefore, minimizing contamination from field evidence collection through the forensic DNA laboratory analysis is imperative for DNA profile interpretation. Enforcing strict use of personal protective equipment, such as wearing laboratory gloves, can decrease rates of technician-mediated contamination of evidentiary objects and cross-contamination between evidentiary items. This study investigated the possibility of contaminating laboratory gloves after prolonged box use in a variety of laboratory settings.

The study was conducted in two phases: (1) to assess whether the removal of gloves from both new and previously opened boxes of gloves by gloved and ungloved researchers transferred DNA to the gloves or glove box ( $n=15$ ), and (2) to assess the presence of transfer DNA on used and open glove boxes found in active academic and research laboratories ( $n=30$ ). Samples from both stages of the study were extracted and amplified using standard forensic DNA laboratory procedures at two analytical thresholds to assess the presence of DNA at increasingly sensitive levels.

In the first phase, only one sample taken from an open box of gloves yielded results at three loci. In the second phase, three samples collected from boxes in academic laboratories yielded quantifiable DNA. Two of these samples generated partial profiles at both analytical thresholds, and the third only produced results at the Amelogenin locus. However, none were from boxes in laboratories with strict contamination precaution procedures in place.

This study found no evidence of manufacturer contamination in the samples taken from unopened boxes of gloves marketed as sterile products. Therefore, the DNA detected on the gloves and boxes in this study was likely introduced by individuals in and around the laboratory.

The introduction of trace DNA from gloves could potentially complicate the interpretation of DNA typing results in cases of low-level profile mixtures, as is usually the case with forensic samples. However, by identifying potential routes of DNA transfer during the handling of evidentiary material, laboratory procedures can be implemented to reduce the risk of technician-mediated contamination.

The results of this study indicate that although contamination of laboratory gloves is possible through regular use, forensic laboratories with restricted access and strict cleanroom protocols may already have the appropriate measures to reduce this potential source of error.

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#### Forensic DNA, DNA Transfer, DNA Contamination



### **F10 Post-Conviction DNA Testing: A Laboratory’s Perspective and Participation in a Multi-Agency Collaboration for the Identification and Evaluation of Post-Conviction Cases**

*Kerry A. Collins, JD\**, Massachusetts Department of State Police, Maynard, MA 01754; *Lynn A. Schneeweis, MS\**, Massachusetts State Police Crime Laboratory, Maynard, MA 01754; *Dorothea S. Collins, MLA*, Massachusetts State Police Crime Laboratory, Maynard, MA 01776; *Kristen Sullivan, MS*, Massachusetts State Police Crime Lab, Maynard, MA 01754

**Learning Overview:** After attending this presentation, attendees will understand the useful contributions a laboratory can make, outside of performing forensic analysis, in the assessment of cases for potential post-conviction testing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a model for collaborative, multi-agency efforts to identify cases potentially suitable for post-conviction testing and address logistical challenges encountered therein.

In 2012, Massachusetts passed Chapter 278A, a post-conviction DNA and forensic analysis law. As a result, in 2013, five criminal justice agencies within Massachusetts—the Committee for Public Counsel Services (CPCS), Middlesex County District Attorney’s Office (MCDAO), Middlesex Superior Court Clerk’s office, Suffolk County District Attorney’s Office, and New England Innocence Project (NEIP)—formed the Massachusetts Working Group (WG) on Post-Conviction Testing Assistance. This working group received funding through a Post-DNA Testing Assistance award for identifying DNA-based innocence claims in serious violent felonies, locating and testing evidence in said cases, and adopting best practices for inventory and storing evidence. In 2014, the working group recognized the potential benefits of expanding this partnership and invited the Massachusetts State Police Crime Laboratory (MSPCL), Boston Police Laboratory, and Suffolk County Superior Court Clerk’s office to join. The newly expanded working group subsequently received funding under the 2014 Department Of Justice (DOJ) Post-Conviction Testing of DNA Evidence to Exonerate the Innocent. The Working Group’s goal for these funds included conducting comprehensive evidence inventories in participating counties, creating a “best practices” guide for evidence management, and conducting a review of pre-2000 cases at MSPCL where microscopic hair comparison was performed.

The MSPCL’s initial contribution to this project focused on providing technical expertise on evidence handling and storage procedures. Laboratory personnel served as resources for police departments conducting evidence room inventories and provided guidance for inquiries as to improving the storage and packaging of pieces of evidence previously subjected to less than ideal conditions. Additionally, the MSPCL provided technical resources for the construction of a “best practices” guide for evidence management to assist the legal community.

The primary role MSPCL undertook in this project was to identify forensic cases within MA where microscopic hair comparisons had been performed and resulting associations between items of evidence and known samples were made. Criteria were determined by the WG to prioritize which categories of these cases would be identified for further review by CPCS and NEIP to assess for potential post-conviction DNA testing. A 20-year time frame of cases for review was established and over 20,000 serology cases were administratively screened to determine those in which hair was examined and associations were made.

During this project, the WG convenes regularly to evaluate progress toward these goals. Through this collaboration, the WG expanded the scope of their work to include: training on post-conviction cases for attorneys; developing a post-conviction template motion for DNA testing; drafting a discovery materials agreement between the MSPCL, MCDAO, and CPCS; and creating a DNA technology timeline for attorneys litigating post-conviction cases.

This presentation will include discussion of the achievements of the Working Group’s efforts over the past five years since its inception. Specific emphasis will be on the role of the MSPCL’s collaboration with the legal community in the identification and evaluation of the suitability of cases for post-conviction DNA testing.

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#### **Post-Conviction, Multi-Agency Collaboration, DNA Testing**



### F11 False Confessions in Juveniles: Do Juveniles Know and Understand Their Miranda Rights?

*Crystal Cafiero, MFS\**, Carlsbad Police Department, Carlsbad, CA 92065; *Ismail M. Sebetan, MD, PhD\**, National University, La Jolla, CA 92037-1011; *Paul Stein, PhD\**, National University, La Jolla, CA 92037

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**Learning Overview:** After attending this presentation, attendees will better understand the importance of providing juveniles with knowledge about the Fifth Amendment. Statistics of false confessions pertaining to juveniles will be examined. As a result, this presentation will highlight the importance of educating youth (and their caregivers) about their constitutional legal rights.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying factors that can cause false confessions and will examine different ways that can assist law enforcement personnel to prevent unknowingly obtaining a false confession. This presentation will look at possible procedural changes in law enforcement policy and how the community can work together to lower that risk of false confessions and reduce the economic impact of these. The study also examined whether there were differences in gender, as well as an understanding of the meaning of the term “in custody” between adults and juveniles.

This study, conducted via social media, was an internet-based survey of 100 adults above the age of 18 years, who were parents or guardians of a child and were asked whether they were aware of the different portions of the Miranda warning. It also asked if they believed the children under their care understood the same. The online survey was sent out via social media with the survey being conducted on Survey Monkey®. Participants were asked eight questions related to the Miranda warning and two demographic questions. Results to the survey questions were analyzed by a chi square test, and statistical significance was determined by *p value* <0.05 to the related survey questions.

Results showed that there was no significance difference between adult males and females when it came to what they understood regarding their children’s rights. When comparing the difference between men and women and their knowledge of juveniles being given food, water, or rest during an interrogation, there was no statistical difference ( $p>0.05$ ). When comparing men and women with the knowledge that the questioning must stop if the child asks for a parent or lawyer, there was a marginal difference ( $p=0.055$ ). The third hypothesis compared the difference between men and women and their knowledge that a parent does not need to be notified of an interrogation, which also showed no significance ( $p>0.05$ ). The last hypothesis tested was the difference between adults knowing the meaning of “in custody” and what they believed their children understood. There was a significant difference in this hypothesis ( $p=.001$ ). Children not understanding their rights could significantly raise the number of false confessions to crimes they did not commit. False confessions are a serious problem in our justice system, leading to tax dollars being spent on trials and incarcerations that should not happen, and possible lawsuits down the road for missteps taken during the investigation and trials. The findings of this study show that there is a much work still to be done in this field to find a solution to lowering false confessions.

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#### False Confessions, Juveniles, Miranda Warning



### F12 A New Informed Consent in View of the 2017 Italian Law 219—Marketing and Communication

*Valentina Ronco, MD\**, Section of Legal Medicine D.I.M., Bari 70124, ITALY; *Fiorenza Zotti, PhD*, Bari 70124, ITALY; *Angela Pezzolla, Bari, ITALY*; *Eloisa Maselli, MD*, Bari 70122, ITALY; *Valeria Bruno, MD, D.I.M.*, Bari, Puglia 70124, ITALY; *Francesca Donno, MD*, University of Bari, Bari 70121, ITALY; *Davide Ferorelli, Bari, ITALY*; *Gianni De Giorgio, Section of Legal Medicine, Bari, ITALY*; *Federica Misceo, Bari, BA 70123, ITALY*; *Alessandro Dell'Erba, PhD*, Risk Management Unit, Bari 70124, ITALY

**Learning Overview:** After attending this presentation, attendees will understand that informed consent is a prerequisite for exercising the medical arts and is based not only on the law, but also on professional ethics.

**Impact on the Forensic Science Community:** This presentation will have an impact on the forensic science community by showing that a new type of informed consent is possible as well as desirable.

The Italian Constitution primarily recognizes and protects the inviolable rights of the person, including individual freedom and health, guaranteeing the citizen the respect of his free choice in terms of health treatments that he may, or may not, be required to undergo. Regarding informed consent, the 1992 National Bioethics Committee document “Information and Consent to the Medical Act” outlined its characteristics. The prevailing powers of jurisprudence define it as “a fundamental principle in terms of protection and health ... a real entitlement of the person in question.” On a national and European level, adequate information about health treatment is recognized and guaranteed in which every subject must provide a valid and preventive consent. Considering this, it is imperative to recall the legislative text No. 219 of December 2017, which, in Article 1, expresses itself in terms of informed consent and underlines how much the patient-doctor relationship is based on an individual’s self-determination.

A prospective study was carried out in collaboration with the Video Laparoscopic Surgery Department of the Policlinico of Bari and the University of Liverpool. The goal was to verify both the effectiveness and efficiency of both medical and legal purposes, being a consensus constructed according to the rules of marketing and communication.

The study includes the sampling of 54 patients undergoing laparoscopic cholecystectomy from April to June 2018. After formalization of their willingness to participate in the study, patients were randomized into the control arm and given the classic form for informed consent to the surgical intervention and experimental arm for the study of the innovative module. At discharge, a customer satisfaction questionnaire was administered, consisting of different items, all sharing an evaluative purpose, with the goal of highlighting whether the information included during the pre-operative phase was better conveyed by the classic or experimental questionnaire.

Fifty-four patients who had undergone laparoscopic cholecystectomy (29 females, 25 males) were recruited. Their average age was 53 years. The sample presents a level of education that is equally distributed (high school diploma 45%, college degree 55%). There was no statistically significant difference between the two groups by index of understanding ( $p=0.5568$ ), clarity of definition of the pathological process ( $p=0.6702$ ), or patient satisfaction ( $p=0.7062$ ). Subsequently, the analysis shifted to the interpretation of the Likert scale, both by evaluating the response trend within the general population and by evaluating the response within the two arms of the study, independently. It became evident that in total, without discerning between control and experimental arms, the study population considered the issue of giving consent to be a useful, clear, crucial tool for their condition as a patient.

The results (even considering the small size of the analyzed sample) demonstrate the equivalence of the two modules for both content and clarity. It is undeniable that the new, more concise, dynamic module, developed by a marketing expert, led to a more marked appreciation from those to whom it had been submitted. Therefore, modernizing the module’s tool could be a starting point from which to reduce the distance between the practitioner and the patient.

#### Consent, Marketing, Communication



### F13 *Voluntas Aegroti Suprema Lex Esto: Ethics in Real Life in New Italian Law*

Francesca Donno, MD\*, University of Bari, Bari 70121, ITALY; Valentina Ronco, MD, Section of Legal Medicine D.I.M., Bari 70124, ITALY; Davide Ferorelli, Bari, ITALY; Gianni De Giorgio, Section of Legal Medicine, Bari, ITALY; Fiorenza Zotti, PhD, Bari 70124, ITALY; Eloisa Maselli, MD, Bari 70122, ITALY; Valeria Bruno, MD, D.I.M., Bari, Puglia 70124, ITALY; Loreto Gesualdo, FP, Regional Transplantation Puglia Center, Bari 70124, ITALY; Alessandro Dell'Erba, PhD, Risk Management Unit, Bari 70124, ITALY

**Learning Overview:** After attending this presentation, attendees will be aware of the possible ethics rather than legal conflicts between the need to respect the patient's health self-determination and the role of the National Transplantation Commission that must guarantee the national health security.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a framework to balance the latent conflict between the right of the individual to refuse a treatment and the community interest to protect the national health.

Spirituality and religiosity are often underestimated, but they are influential factors in Western medicine, as demonstrated by Jehovah's Witnesses' experiences and their "religious restrictions" in the approach to the care system.<sup>1</sup>

There are studies in literature moving beyond "dyadic relationships" and caregiving contexts and contribute to a deeper understanding of care and relational conceptualizations of Witness patients' autonomy globally.<sup>1,2</sup>

In the Italian national context, the promulgation of Law 219, issued on December 22, 2017, "*Norme in materia di consenso informato e di disposizioni anticipate di trattamento*" (GU General Series n.12 on January 16, 2018) has modified the basic framework related to these topics by a systematic regulation of informed consent concerning the diagnostic-therapeutic practices and the introduction of the new institute of "*Disposizioni Anticipate di Trattamento*" (DAT—the Italian for Advance Healthcare Directive (AHD)—also known as a living will) as well as that referring to the "shared planning" of cure.

The L.219/2017 states the overcoming of Hippocratic ethical-deontological tradition: the patient's conscious and freely expressed will becomes the nodal point of the lawfulness of the medical-surgical treatment. Translating this innovative forensic matter in the field of transplant medicine is an essential operation.

This study conducted a critical analysis according to traditional criteria of the "triptych" legal medicine/ethics/law of a case received by the Regional Transplantation Puglia Center and its Third Commission. The story refers to a pair of siblings: the receiver is a man, 53 years old, suffering from chronic renal failure who has been in dialysis for three years. The most compatible donor is a 51-year-old female in the family affected by thalassemia trait. She is a Jehovah's Witness and therefore rejects blood transfusions because of her religious belief.

The case presented is a paradigmatic example of the latent conflict between the right of the individual and the community interest: the patient/receiver versus the donor/bearer of rights. The setting is in the frame of a public health national service, with its needs, health workers' rights/duties, and the economic limits and related allocation of resources according to the principle of the maximum benefit.

The resulting and essential question is: Who should be first? The patient, the donor, and/or the global "system?" Which criterion and scientific method should be used to harmonize the whole system to respect basic and basilar standards? And, mainly, can the risk intrinsically linked to medicine be overcome unilaterally? The transplant community will have to reformulate the method, criteria, organization, and routine procedures through a deep analysis about ethical-deontological assumptions and "new" normative provisions resulting in a concretely useful compromise.

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**Ethics, Transplantation, Advanced Healthcare Directive**



### F14 The Potential and Problem of Academics as Forensic Experts

Carole E. Chaski, PhD\*, ALIAS Technology, LLC, Georgetown, DE 19947

**Learning Overview:** After attending this presentation, attendees will be able to evaluate academics for the role of forensic experts and analyze the academic Curriculum Vitae (CV) for trustworthiness and exaggeration.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an argument for regulating academic experts and ways for evaluating the academic expert CV.

The role of academics as expert witnesses is full of potential yet fraught with problems. The potential is great. First, an academic with a doctorate can have an exceptional amount of knowledge in the general field, particularly in the areas of specialization as reflected in research grants and publications. Second, academics can discern differences between their own and another field's methods and discern when methods are being properly applied or not. Third, academics know the controversies and unsolved problems in their field. Fourth, academics know how different groups relate to "schools of thought" within their fields and the relative seriousness with which different schools of thought are taken. Fifth, academics can know specific technical details related to these schools of thought (e.g., arcane controversies, such as the proper/improper use of particular statistical procedures or stances regarding the use or non-use of data types or requirements for the doctorate). Sixth, academics know the respected, prestigious conferences and journals in their fields. Seventh, since most academics regularly lecture, an academic can express himself easily, especially if the academic teaches introductory courses as well as upper-level and graduate seminars.

Scandals concerning forensic science have focused almost exclusively on bad behavior within crime laboratories. These bad behaviors include falsified credentials, mishandling of evidence, etc. and are detectable because the crime laboratories are public facilities. However, the regulation of academics as forensic expert witnesses is left for a case-by-case basis, using the Rules of Evidence and evidence standards such as *Daubert*.<sup>1,2</sup> The case-by-case method may not be enough for two reasons. First, the hierarchy of doctorates shows that lawyers, as well as the jury, are to a degree intimidated by academic doctorates. Common parlance shows this: MDs are known as "real doctors," PhDs are known as "doctors," and JDs are called "lawyers." Thus, lawyers and judges may not feel qualified to evaluate academic experts realistically. Second, *Daubert* criteria are not applied consistently from case to case. One expert can be allowed to testify in one jurisdiction and not be allowed to testify in a different jurisdiction, even though the academic expert's credentials and testimony are essentially the same.

The forensic science community should look closely at regulating academics as forensic experts. Each potential has a downside, with eight additional problematic factors. First, many academics feel that they are not paid enough as professors. The primary motivation for consulting may be greed. Second, many universities seeking ways to be "relevant to the community" encourage faculty to supplement their incomes with expert witness consulting. Third, this approach brings media attention to the university and the expert, often during a case. If the academic wants to please his university masters, publicity is his friend, even if this publicity displeases his attorney masters. Fourth, this dual-income approach may be questionable as "double-dipping" from government coffers. Fifth, this dual-work approach will interfere with either the academic's teaching and publishing/writing schedule or the attorney's case schedule; one will suffer. Sixth, the academic's publications may change from actual research to case studies of the cases. Seventh, the case work may actually be performed by (under)graduate students rather than the professor. The confidentiality agreement usually does not extend to (under)graduate students since the attorney assumes that the academic expert is going to do the work. Eighth, the CV may reveal problems to other academics but not to lawyers. It may be necessary to hire another academic in the same field to review the CV, to have a chronological series of the academic's CV to reveal an illogical timeline of "worked cases." Tricks on the academic CV are presented.

Finally, this talk concludes with potential regulations for academics as experts so that the judicial system can be afforded all the benefits of academic expertise while avoiding the pitfalls during trial.

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#### Academics, Expert Witnesses, How to Read Academic CV



### F15 The Duty to Correct Erroneous or Inadvertently Misleading Forensic Science Testimony

*Sarah Chu, MS\*, Innocence Project, New York, NY 10013; Bryce Benjet, JD, Innocence Project, New York, NY 10013*

**Learning Overview:** After attending this presentation, attendees can expect to be better informed regarding circumstances in which testimony should be corrected and how it can be corrected.

**Impact on the Forensic Science Community:** Forensic science practitioners, service providers, and medical service providers have an active, rather than passive, duty to correct as scientists. This ethical duty requires scientists in the system to take proactive action when testimonial problems are identified, rather than await permission from legal actors of the system. This presentation will impact the forensic science community by providing examples of how this type of testimony may occur and provide support for solutions for addressing the problem.

Most post-conviction accountability schemes for correcting forensic science testimony problems and notifying the affected defendants are predicated on the issue rising to the level of: (1) negligence or misconduct, or (2) the misapplication of forensic science in which testimony exceeds the limits of the science. This presentation will discuss a third and less-frequently discussed category of forensic testimony problems—inadvertently or unintentionally misleading testimony.

Wrongful convictions can result from inadvertent mistakes—such as a sample switch—or from testimony that unintentionally becomes misleading based on proceedings of the trial that are beyond the forensic science practitioner’s control. It is acknowledged that forensic scientists routinely provide testimony in isolation, and attorneys will raise questions in their direct and cross examinations in pursuit of a trial theory that may not reflect ground truth or sound science. The forensic science practitioner, who may be the only party in the courtroom transactions who understands the scientific limitations of the forensic evidence at hand, may be offering expert testimony and providing honest and technically correct answers, but unknowingly imparting information that can mislead fact finders in a case. The impact of this inadvertently or unintentionally misleading testimony may be apparent to the forensic science practitioner during the case or may not be revealed until the hindsight of postconviction proceedings. When a forensic science practitioner’s testimony is misused, rendered misleading, or inadvertently mistaken, what can be done to support the expert in correcting the record?

Because there is currently no national oversight framework for Forensic Science Service Providers (FSSPs) and Forensic Medical Service Providers (FMSPs) in the United States, the criminal justice system relies on a patchwork of laboratory accreditation and state-level regulation to hold FSSPs accountable. Neither of these systems is comprehensive and complete as FSSPs and FMSPs continue to operate without accreditation and few states have a system in place to fully oversee the activities of FSSPs and FMSPs. Consequently, we rely on the forensic science community to self-regulate and we need it to have the capacity to correct problems regardless of the presence or absence of formal regulatory systems. Most existing accountability schemes for forensic science testimony, such as state forensic science commissions and Coverdell investigations, limit the scope of their duty to correct to cases where negligence or misconduct is ascertained. Categorizing problems in such a manner often requires a finding of fault or error that create barriers to comprehensively assessing testimonial problems and have no bearing on guilt or innocence. This study submits that the standard of care for forensic science systems to correct inadvertently or unintentionally misleading testimony is an ethical duty and is under-recognized because the most prominent cases of testimony correction are based on the identification of negligence, misconduct, or misapplication. The latter system is not a solution for addressing wrongful convictions as many forensic problems arise unintentionally. Forensic science accreditation guidance suggests that a forensic scientist try to prevent misleading testimony on the stand; both the guidance material and a change in science legislation facilitate methods of correction when a forensic science practitioner is made aware of problems arising from his/her testimony after the fact.

However, because forensic scientists may move on professionally or because they are typically removed from the litigation process, this presentation will demonstrate that requesting letters of correction from the institutional FSSP or FMSP when testimonial problems occur would be an additional method for fulfilling the forensic science practitioner’s duty to correct and notify. This presentation will use actual communications with FSSPs and an FMSP to illustrate how forensic science and medical practitioners and service providers approach the duty to correct problems with testimony. (This presentation will redact names of individuals from communications presented.)

#### Duty to Correct, Ethics, Testimony





### F16 The Ethical Boundaries of Presenting Expert Testimony in a Criminal Trial

*Walter M. Reaves, JD\*, Law Office of Walter Reaves, PC, Waco, TX 76701*

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**Learning Overview:** The goal of this presentation is to address an issue that has been observed in the use of experts in criminal cases: the use of experts with minimal experience or knowledge of the subject they are testifying about, or experts who testify about subjects that are outside their area of expertise. Examples will be provided of cases in which testimony has been allowed, and the impact that testimony has had on cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing the ethical issue of experts with insufficient experience or knowledge of the subject about which they are testifying.

This presentation is designed to raise awareness and generate discussion about the ethical obligations of forensic experts and attorneys when presenting testimony in a criminal trial.

The criminal justice system is an adversarial one. Both sides have the obligation to zealously represent their clients. A prosecutor has the added obligation to ensure that justice is done. That concept is easily stated, but harder to apply. At the outer limits, a prosecutor is prevented from presenting evidence they know to be false. In some jurisdictions, this limitation extends to evidence that would mislead the jury or encourage a false impression. Courts have been reluctant to find that line has been breached, and disciplinary authorities are even more reluctant to impose sanctions. The result is that the limits of zealous advocacy can vary from jurisdiction to jurisdiction and even between prosecutors in the same jurisdiction.

While this is always a potential problem, there are special concerns when experts are used. Jurors rely heavily on experts, assume that the prosecutor has researched their credentials, and believe they are qualified. Judges often rely far too heavily on prosecutors and routinely allow experts to testify. While the ultimate decision is up to the judge, the practical impact is that the State decides which experts are qualified. Far too often, little to no research has been done, other than checking with prosecutors, and prosecutors rely on the expert to educate them. While there are now more resources available to defense counsel, far too few defense lawyers take advantage of them. There is also a disparity in resources, and in some fields, there are few experts available to consult with defense lawyers. The result is that experts offered by the State often go unchallenged on both their qualifications and the subject matter of their testimony.

While there are limits placed on lawyers (even if they may be vague), there are even fewer limits on experts. Most experts are members of several different organizations. Some organizations may have ethical guidelines, while others don't. This presentation will explore what limits exist and whether there are any meaningful limitations, or whether each expert is left to his or her concept of what is right. Emphasis will be placed on the obligation experts have to inform prosecutors about the limits of their testimony, as well as what obligations they must meet with defense counsel, and whether there is any obligation to inform the courts about areas in which they may not be qualified.

The goal of this presentation is to encourage discussion and solicit proposals and suggestions to deal with these issues. Reliance on the courts has proved to be of little use and expecting prosecutors to limit the use of evidence that may help obtain a conviction is problematic. The hope is that not only will proposals and suggestions be identified, awareness of the issue will also be increased.

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**Academics, Expert Witness, How To Read Academic CV**



### F17 I Didn't Get It Wrong: Prosecutorial Denial in the Face of Forensic Evidence of Innocence

Alissa L. Bjerkhoel, JD\*, *California Innocence Project, San Diego, CA 92101*

**Learning Overview:** After attending this presentation, attendees will understand case examples in which the prosecution continued to vigorously defend the validity of convictions despite post-conviction forensic evidence showing innocence. This presentation will also cover the trend of prosecutors pursuing endless appeals or offering plea deals even when innocent petitioners have been successful in the courts.

**Impact on the Forensic Science Community:** The presentation will impact the forensic community by serving to identifying a trend in the law enforcement and prosecutorial communities to dismiss evidence of innocence and maintain tunnel vision in re-examining old cases. Several case examples will be discussed.

“These two bastards are guilty. I just can't prove it” –Jefferson County Assistance District Attorney Arthur Green, commenting on the exonerations of Ronnie and Dale Mahan.<sup>1</sup> And yet, if one cannot prove guilt, how can one be so confident they are, in fact, guilty?

It was 1998. Her body was lying on a pile of rocks on the bank of a prominent local fishing hole. She had been strangled to death. Her bra had been pulled up, exposing one of her breasts. Yet there was no evidence of a sexual assault. She had no drag marks or other injuries, indicating she was not strangled at that location; rather, she was placed there. The desert heat beat down on her for four days until two fishermen found her. A piece of rope (the ligature) and a broken man's wrist watch were found among other debris at the scene. The hunt was on for whoever owned that watch and for whoever could have done this horrific act.

“That's my watch.” Those would be the fatal words of James Rogers during his police interview. James was a coworker of the victim and the two, although separately married, were having an affair. It would turn out that James was mistaken about the watch and he did produce a similar watch to the police, but it was too late. They caught their man. Or did they? Subsequent testing of the watch revealed the profile of the victim's estranged stepson, 19 years old at the time and accused by the victim of molesting her children. His father was going through a contentious divorce with the victim. Further testing and a hit in the Combined DNA Index System (CODIS) revealed a second perpetrator—the stepson's cousin's DNA underneath the victim's fingernail. These results have been known for years and, yet, James remains in prison.

James's case is not unique. Lawyers working in projects and offices dedicated to the release of innocent prisoners have faced backlash from law enforcement and prosecutors, even in the face of seemingly clear-cut forensic evidence of innocence. They have encountered the “unindicted co-jaculator theory,” meaning that unknown semen on a victim can be explained away by simply claiming that there must have been two perpetrators. They have encountered claims that male DNA on a rape victim's clothing must have come from the victim washing the clothing in the same washing machine as the person whose DNA is found on the clothing. They have argued contamination in the crime lab, DNA evidence having come from someone sneezing on the victim's clothing, 8-year-olds being sexually active, someone stumbling across a murder victim's body and engaging in acts of necrophilia. The list goes on. And when that all fails, they have even accused lawyers of planting evidence.

The root cause of prosecutorial denial of innocence has still not been adequately defined. Perhaps it is psychological. It has been said that “[w]hen we are confronted with evidence that challenges our deeply held beliefs, we are more likely to reframe the evidence than we are to alter our beliefs.”<sup>2</sup> Perhaps it is political. Perhaps both. Whatever it may be, it is a flaw in our criminal justice system that does not seem to be getting better.

#### Reference(s):

- <sup>1</sup> Lara Bazelon. *Seventeen Cases of Denied Innocence*. *Slate*. January 10, 2018.
- <sup>2</sup> Matthew Syed. *Black Box Thinking*. 2015.

#### DNA, Innocent, Prosecutor



### F18 K-9 Evidence in the Courtroom

*Mary E. Cablk, PhD\*, Desert Research Institute, Reno, NV 89512*

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**Learning Overview:** The goal of this presentation is to convey what constitutes defensible canine (K-9) training and deployment to non-K-9 experts. Attendees will learn about discovery materials in assessing a K-9, its handler, and the sniff(s), regardless of whether the case pertains to narcotics detection, trailing, or another K-9 discipline.

**Impact on the Forensic Science Community:** The impact of this presentation on the forensic science community is to improve understanding of a complex resource component of law enforcement and forensic investigation resource: the K-9 team.

The use of K-9s in law enforcement for the detection of illicit substances, human remains, crime scenes, and trailing (following the path of travel of an individual person) has a long history. Courts are generally of the opinion that K-9s are reliable. A properly trained K-9/handler team can provide valid, correct, accurate, and defensible information that informs an investigation. The K-9 belonging to an agency is administered as equipment and may be paired with multiple handlers over the course of its career. It is expected to perform reliably regardless of the capability, skill, training, or experience of its handler.

K-9/handler teams are not infallible, and it is generally accepted that K-9 teams are not perfect. How does an expert determine whether a sniff or a trail was reliable? What about the situation in which the K-9 is deployed to find evidence, such as a firearm or narcotics tossed during a pursuit, but none is located? Are all K-9 teams equal? This presentation will address these questions and others.

One role of the expert is to expose weaknesses in the K-9 team from initial training through pairing with a handler, maintenance training, certification, and deployment. Even when there are deficiencies in protocols, policy, and adherence to best practices or industry standards, canine evidence typically prevails in the courtroom.

There has long been a practice of minimal record keeping in the K-9 community with the belief that no fault can be found where no documentation exists. That archaic approach defies the direction and integrity of the forensic sciences today. There is no mystery as to how a reliable K-9 is trained, although pertinent information that is intended to support the reliability of the K-9 team is not conducted or maintained in a consistent, universally accepted manner. Every department has their own policy and there are as many standards as there are agencies and K-9 organizations. This creates opportunities for the expert to find fault and deficiencies within a department, within a unit, and with individual K-9 teams, where there may in fact be a rigorous practice employed, and the teams are truly reliable. As the K-9 community comes to embrace the science that supports proper training and thus produces an unmatched resource to forensic investigation, it will become increasingly difficult to discredit K-9 evidence. More importantly, the usefulness of K-9 evidence to an investigation will move toward meriting scientific support and away from being an art.

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#### Canine, Best Practices, Probable Cause



### F19 Legal Theories for Prosecution of Opioid Overdoses Resulting in Death Under State and Federal Law Principles

M.J. Menendez, JD\*, *Organized Crime Drug Enforcement Task Force, Washington, DC 20530*; Barry K. Logan, PhD, *NMS Labs/CFSRE, Willow Grove, PA 19090*

**Learning Overview:** After attending this presentation, attendees will be able to describe the theories of prosecution underlying state and federal investigations, prosecutions, and sentencing of cases involving single and polydrug overdose death cases. Attendees will also be able to explain the “but for” causation legal precepts set out in *Burrage v. United States* and describe how the Burrage case and its progeny apply to the federal, state, and local theories of prosecution.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting understanding and discourse on the criminal justice systems’ response to deaths caused by the opioid epidemic at local, state, and federal levels, providing attendees with a foundation to assess the deterrence theory of prosecution against other societal responses.

Per the Centers for Disease Control and Prevention (CDC), the rate of synthetic opioid overdose deaths in the United States rose from 3.1 to 6.2 deaths per 100,000 between 2015 and 2016, marking the first year that synthetic opioids became the most common type of opioid involved in overdose deaths. CDC’s statistics also report that more than 55% of opioid overdose deaths occurring nationally in the 12-month period ending November 2017 involved synthetic opioids, accounting for more than 27,000 overdose deaths. Against this stark tragedy, public health and public safety officials grapple with appropriate responses. Increasingly, federal and state law enforcement agencies and prosecutors have charged distributors of the drugs causing overdoses and overdose deaths. Federal prosecutors, working under *Burrage v. United States* and its progeny, as well as under the United States Sentencing Guidelines, focus investigation and prosecution efforts on death resulting from the distribution of a substance and “but for” causation.<sup>1</sup> State and local prosecutors charge a wide variety of offenses with varying mental states and proximate cause nexus requirements, including but not limited to First, Second, and Third-Degree Murder; Voluntary, Involuntary, and Reckless Manslaughter; Drug-Induced Homicide, Drug Delivery Resulting in Death, with attendant inchoate theories. Amid these decisions, the holding of *Burrage v. United States* stands, but the question of its applicability and limits under the various state charging theories lingers.<sup>1</sup>

This presentation will provide a classification overview of state prosecution theories for drug-involved overdose deaths, with particularized discussion of culpable mental states, proximate cause and nexus of drugs to death, and polydrug deaths as interpreted in judicial cases. The overview will enable attendees to argue the factual and legal nuances that cause seemingly irreconcilable outcomes among cases and related legal proceedings. The discussion will then highlight federal cases interpreting the 21 U.S.C. Section 841 “death resulting” enhancement, as well as United States Sentencing Guidelines amendments from 2018 affecting charging theories.<sup>2</sup> This presentation will conclude with a hypothesis for synthesizing the various theories of prosecution and their respective attendant penalties, allowing participants to assess the propriety of prosecuting overdose deaths in the various levels of the criminal justice system.

#### Reference(s):

<sup>1</sup> *Burrage v. United States*, 571 U.S. \_\_\_\_ (2014).

<sup>2</sup> 21 U.S.C. § 841—U.S. Code—Unannotated Title 21. Food and Drugs § 841. Prohibited acts A.

#### **Burrage, Overdose Death, Prosecution**



### F20 Novel Psychoactive Substances, Fentanyl Analogues, and Scheduling: Federal and State Responses

*M.J. Menendez, JD\**, Organized Crime Drug Enforcement Task Force, Washington, DC 20530; *Barry K. Logan, PhD\**, NMS Labs/CFSRE, Willow Grove, PA 19090

**Learning Overview:** After attending this presentation, attendees will understand the temporary scheduling provisions of the United States Code and explain how the temporary scheduling factors were utilized to affect the scheduling of all fentanyl-related substances. Attendees will also gain familiarity with the methodology for various state law scheduling constructs as they relate to federal scheduling. Finally, attendees will learn of the most recent judicial authority interpreting the temporary scheduling and emerging novel psychoactive substances.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by synthesizing statutory and judicial authority relating to novel psychoactive substances and fentanyl analogues, as well as highlighting the challenges in interpretation and application that are endemic to the legal analyses.

The supply, distribution, and potency of illicitly manufactured fentanyl analogues and other illicit opioids in the United States drug markets continues to evolve. According to the Centers for Disease Control and Prevention's Health Alert Network report (November 7, 2018), seized drug submissions testing positive for fentanyl or fentanyl analogues were numbered at 25,460 reports in the first six months of 2017. Many states saw fentanyl or fentanyl polydrug deaths outnumber heroin deaths in 2017. The sophisticated testing methodologies and equipment required to detect and identify novel fentanyl analogs, combined with the rapid evolution in the structure of fentanyl analogs and novel psychoactive substances, continue to challenge and hinder law enforcement and public health responses into present day 2018.

In February 2018, the Acting Administrator of the Drug Enforcement Administration ordered temporary placement of all fentanyl-related substances into Schedule I of the Controlled Substances Act based on a finding that placement of the synthetic opioids into Schedule I was necessary to avoid an imminent hazard to public safety.

This presentation will present the legal framework for temporary scheduling under 21 U.S.C. Section 841, with particularized discussion of the legal reasoning and justification for scheduling all fentanyl-related substances.<sup>1</sup> The number of fentanyl analogs, illicit opioids, and novel psychoactive substances identified since the fentanyl-related substances announcement will be presented to engender discussion of the impacts of the order scheduling fentanyl-related substances. This presentation will then feature classifications of state law statutory and regulatory amendments resulting from the Drug Enforcement Administration's (DEA's) scheduling of fentanyl-related substances.

Fentanyl-related scheduling has also impacted federal legal precepts in charging, litigating, and sentencing fentanyl analogs. An overview of the judicial, statutory, and regulatory changes in federal law will be provided, with case studies on recent decisions interpreting the relationship and differentiation between the terms fentanyl analog and analog of fentanyl in the evolving legal landscape. The United States Sentencing Guidelines amendments from 2018 affecting fentanyl analogs and other novel psychoactive substances will also be presented.

**Reference(s):**

<sup>1</sup>. 21 U.S.C. § 841—U.S. Code—Unannotated Title 21. Food and Drugs § 841. Prohibited acts A.

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**Fentanyl, Analogue, Scheduling**



### F21 Secure Continuous Remote Alcohol Monitor (SCRAM®) Test Results: Fact, Fiction, Puffery, Rhetoric, and Hokum

Donald J. Ramsell, JD\*, Wheaton, IL 60187; Gil Sapir, JD, Chicago, IL 60680

**Learning Overview:** After attending this presentation, attendees will understand the necessity of questioning SCRAM®-purported Transdermal Alcohol Concentration (TAC) test results when used to enforce alcohol abstinence, as it is now commonly used within the legal community.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing recognition of what may constitute marketing rhetoric regarding the accuracy and reliability of biomonitoring alcohol devices for use within the judicial system to establish court-ordered abstinence.

SCRAM® is designed to measure alcohol content as it diffuses through a person's skin as insensible perspiration. SCRAM's® operation is premised upon measuring volatile substances passing through the skin and alcohol excretion in human perspiration.<sup>1</sup> The device is manufactured by Alcohol Monitoring Systems, Inc. The device, worn as an ankle bracelet, is commercially available to law enforcement agencies and privately operated correctional institutions. It is primarily designed and marketed for court-ordered alcohol monitoring of TAC readings.<sup>2</sup>

SCRAMs® do have limitations. TAC does not directly correlate to Blood Alcohol Concentration (BAC) in a SCRAM®.<sup>3</sup> SCRAMs® are useful in general population biomonitoring of self-induced alcohol consumption as a passive preliminary testing device. Based upon the manufacturer's criteria, SCRAM® can only reliably detect the consumption of five or more standard beers or drinks, and 45.9% of all occasions of drinking one to three beers went undetected when using SCRAM's® 0.02g/dl as a threshold.<sup>4,5</sup> This presentation briefly reviews the manufacturer's material and the scientific literature for factual comparisons of marketing statements and purported test results.

Judges control the determination of "good science" and "evidential reliability" under *Daubert*.<sup>6</sup> Too often products are selected based on their price, convenience, and promotional marketing. In certain judicial proceedings, the standards for reliability of scientific evidence are reduced (i.e., probation violation hearings, parole revocation hearings, bond and bail hearings).<sup>7</sup> SCRAMs® are subject to lower standards of evidential proof. The misuse of scientific devices through dilution questions their appropriateness for legal responsibility.

SCRAMs® are not amenable to the same consistent and evidential alcohol measuring devices, such as breath alcohol instrumentation and gas chromatographs. SCRAMs® are not subject to equivalent uniform standards and regulations for approval, use, maintenance, and calibration, as are other alcohol detecting and measuring devices.<sup>8</sup> The units can be purchased without the same governmental oversight, and there are lower standards than those promulgated in the Driving Under the Influence (DUI) industry. SCRAM® presents selective marketing data and does not adhere to governmental or industry standards for reliability. Initial concern is whether the SCRAM® device is accurate and reliable to identify and measure TAC.<sup>5,9</sup>

Information provided by the SCRAM® manufacturer, published studies, court testimony, and reports from the National Highway Traffic Safety Administration (NHTSA) indicate that the transdermal devices cannot reliably detect levels of alcohol use from one to four (and perhaps up to six) drinks.

Arguably actual limitations of SCRAM® devices and TAC data is skewed or specious through marketing and other claims, which include, but are not limited to, claims stating: (1) it can detect levels of alcohol. Manufacturer information, published articles, court testimony, and the NHTSA indicate TAC devices cannot reliably detect levels of alcohol use from one to six drinks; (2) the SCRAM® device "eliminates testing gaps—no ability to miss a test or drink around testing schedules"; (3) "99.3% of clients (of SCRAM®) are completely sober and compliant every day"; (4) "the SCRAM® device has a greater deterrent effect than other monitoring devices"; (5) "SCRAM® CAM is calibrated using the same court-accepted methodology as evidential breath testing equipment"; (6) SCRAM® CAM has "single-source admissibility—no back up tests required"; and (7) device "conclusively distinguishes alcohol consumption vs. environmental alcohol sources."<sup>5,9-15</sup>

SCRAM's® claims are plausibly misleading or lack sufficient supporting evidence. It appears the use of SCRAM® beyond its intended judicial purpose is problematic. Questionable marketing claims underscore the need for vigilant implementation of good science and scrutiny by the consumer. SCRAM's® marketing of TAC results should not be considered scientifically credible or reliable.

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2. Marques, P.R. A.S. McKnight and NHTSA DOT HS 810 875. *Evaluating Transdermal Alcohol Measurement Devices*. Final Report—November 2007.
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5. N.P. Barnett et. al. Predictors of Detection of Alcohol Use Episode During a Transdermal Alcohol Sensor. *Exp. Clinical Psychopharmacol*, 2014 February; 22(1):86-96.
6. *Daubert v. Merrill Dow Pharmaceuticals, Inc.*, 509 U.S. 579, 593 (1993).
7. See, e.g. *Bell v. State of Florida*, Appellee. No. 5D14–1569. Decided: October 09, 2015.

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### **SCRAM®, Alcohol Testing, Abstinence**



### F22 Due Process and Ethics: Crime Laboratories Cannot Be Paid for Convictions

Michelle L. Behan, JD\*, Behan Ramsell P.L.L.C., Tucson, AZ 85718; Gil Sapir, JD, Chicago, IL 60680

**Learning Overview:** After attending this presentation, attendees will understand the necessity for identifying, exposing, and mitigating bias due to crime laboratory contingency fee revenue.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that contingency fees paid to laboratories upon conviction are unethical and unconstitutional.

Crime laboratories are bound by legal and scientific standards and a code of ethics to foster integrity, honesty, competency, and public trust. It is a violation of ethical canons, and substantive due process, for laboratory personnel to testify in criminal cases when they receive contingency fees should the defendant be convicted. Government crime laboratories were established to perform objective, reliable analysis to assist the justice system. Conscious and subliminal bias exist in crime laboratories, especially when exclusively controlled by law enforcement.<sup>1</sup> This relationship creates a pro-prosecution culture, with scientists an extension of law enforcement. Contingency fees paid to crime laboratories fosters additional bias and adversely affects both substantive due process and ethics. Public confidence in the justice system and integrity of evidence is paramount. It is eroded when fundamental fairness and trustworthiness are degraded or compromised.

Crime laboratories submit their budgetary requests to the state for funding. The legislature enacts revenue statutes for imposition of fees upon conviction. Currently, 17 states have statutes authorizing contingent fees paid to crime laboratories.<sup>2</sup> These fees are collected by the court and forwarded either to the crime laboratory or state fund for redistribution.<sup>2</sup> Contingent conviction fees vary by state and generate enormous revenue.<sup>3</sup> This revenue augments state crime laboratory funding. The revenue creates financial and ethical conflicts for laboratory personnel because their objectivity is compromised. Redistribution from a general fund does not remove the conflict; crime laboratories still receive the revenue. Privately funded laboratories may conduct forensic analysis. Unlike government crime laboratories, they are generally liable for the work product.

The laboratory director determines the facility's budget and distribution of funds within the laboratory. The laboratory personnel's testimony is indelibly tainted through their vested interest in securing funding for their laboratory and employment. Model ethical guidelines from professional scientific organizations prohibit any behavior which "diminish(es) confidence" in the laboratory's "competence, impartiality, judgment, or operational integrity."<sup>4</sup>

The 2009 National Academy of Sciences Report recommended mandatory adherence to a code of ethics, with enforcement and sanctions for violations<sup>1</sup>. However, laboratories may establish their own voluntary protocols and code of ethics. The International Organization for Standardization, the American Society for Crime Lab Directors/Laboratory Accreditation Board (ASCLD/LAB), and other organizational guidelines are voluntary and without viable sanctions.<sup>4</sup>

The Model Code of Professional Responsibility of the American Bar Association (ABA) prohibits witnesses from being "bribed or offered compensation that is contingent on the witness's testimony or the result in the litigation."<sup>5</sup> Forensic scientists are expected to be unbiased and objective and may not participate in any case where a contingency fee is collected. Ironically, scientists who are in violation of ethical canons may still testify. It is unconstitutional for a court budget to be funded in whole or in part by the funds collected upon conviction. The ABA prohibits fines, fees, and penalties from being appropriated to any local unit of government that supports a court.<sup>6</sup>

Criminal defendants have a constitutional right to a fair trial and equal justice. Defendants are deprived of their constitutional rights when a party with a financial or personal interest in the outcome is involved. Courts are expected to protect citizens from statutory legislation that violates due process.<sup>7</sup> Courts and counsel must act to protect the defendant from wrongful convictions based on biased results. Bias is not limited to any discipline. Prohibiting revenue-generating statutes is the appropriate solution.

Attorneys have a constitutional obligation to challenge evidence against the accused. Bias of a witness is never a collateral matter. Attorney competency includes exposing and eliminating bias. Lack of professional memberships, unenforceable codes of ethics, and non-accreditation do not prevent a crime laboratory from conducting and presenting its work product. Crime laboratory contingency fee payments disregard ethical canons of law and science, violate substantive due process guarantees to criminal defendants, and create an unacceptable conflict of interest for forensic scientists. Laboratories cannot be trusted if they have a financial incentive to produce a conviction.

#### Reference(s):

1. National Research Council, Committee on Identifying the Needs of the Forensic Sciences Community, *Strengthening Forensic Science in the United States: A Path Forward*. Aug. 2009.
2. Roger Koppl and Meghan Sacks. *The Criminal Justice System Creates Incentives for False Convictions*, Criminal Justice Ethics, Vol.32, No.2, 126-162 2013; exception is North Carolina which includes private laboratories. The 17 states are: AL, AZ, CA, FL, ID, IL, KS, KY, MS, MO, NJ, NM, NC, TN, VA, WA, WI.
3. Over an 11-year period, Tennessee collected more than \$22 million dollars in contingency fees just from DUI convictions.
4. International Organization for Standardization, ISO/IEC 17025:2017. *General Requirements for the Competence of Testing and Calibration Laboratories*, 4.1.3; *ASCLD/LAB Supplemental Requirements for the Accreditation of Forensic Science Testing Laboratories*, 2011 Ed., Append. B, Guiding Principles of Professional Responsibility for Crime Laboratories and Forensic Scientists, p.31.





## Jurisprudence — 2019

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5. American Bar Association Model Rules of Professional Conduct, 2016, Rule 3.4(b); see also Restatement (Third) of the Law Governing Lawyers, § 117, cmt. D (2002); *City and County of Denver, Colo. v. Board of Assessment Appeals of State of Colorado*, 947 P.2d 1373, 1379 (Colo. 1997) reh'g denied (Dec. 2, 1997).
6. American Bar Association Commission on Standards of Judicial Administration, 107 (1974).
7. Seventeen states permit assessment of crime laboratory fees contingent upon conviction. Only Tennessee held this arraignment to be constitutional under its state constitution. *State of Tennessee v. Rosemary L. Decosimo*, No. E2017-00696-SC-R11-CD (Aug. 23, 2018). No other state has ruled on this practice. The constitutionality however, does not change the ethics of whether this should be permitted. A potential conflict of interest exists. Privately funded laboratories may conduct forensic analysis. Unlike government crime laboratories, private laboratories are generally liable for the work product. North Carolina exempts private laboratories from liability for law enforcement work.

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### **Ethics, Crime Laboratories, Contingency Fees**



### F23 New York City (NYC) Polo Grounds Murders: Was It Bernard Perez or “The Beast”?

*Kerry J. O’Connell, JD\*, The New York County District Attorney’s Office, New York, NY 10013*

**Learning Overview:** The goal of this presentation is to describe the steps taken to meet a claim of psychiatric defense to charges of homicide in a notorious New York City serial killing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing assistance in amassing, preserving, and presenting psychiatric evidence in a criminal trial.

Bernard Perez was convicted by a jury trial, after a host of crimes, for murdering Doris Drakeford and Jerry Pollard in two separate murders. In January 2001, Perez viciously murdered Doris Drakeford so he could take over her apartment in the Dykman Houses in Upper Manhattan, a public housing cluster called The Polo Grounds. At the time, Perez was staying with Drakeford, but when she told him he would have to leave, he decided to kill her. Perez knew that she was a drug addict and was not likely to be missed. Perez strangled her with an electrical cord, wrapped the body in a sheet, stuffed it into a shopping cart, wheeled her to the 207<sup>th</sup> Street bridge, then tossed her and the cart into the Harlem River, where it remained submerged until the spring thaw.

Perez was evicted in late May, just before the body was found, and needed another place to live. Bernard Perez enlisted the help of two friends and struck a second target in The Polo Grounds, Jerry Pollard. After plotting together for a day, Perez realized he should do more than just dump the body, so the murderous trio agreed they would dismember Pollard and dispose of his head and hands in New Jersey. The next day, Perez, Walker, and Williams accosted Pollard in the elevator, dragged him into his apartment, then finished him off. They then proceeded to methodically dismember his body in the bathtub. After they finished the job, Perez invited another friend up to Pollard’s apartment. The visitor saw the dismembered torso in the bathtub and later reported what he had seen to the police. The police later discovered Perez and Williams in the apartment, along with various parts of Pollard’s body wrapped in plastic bags. Other body parts were discovered in bags on the ground outside the apartment and in a nearby dumpster. At the precinct, after waiving his Miranda rights, Perez confessed to killing Pollard and Drakeford. Two of his lengthy, detailed statements were recorded on videotape.

Those statements and others were made after Perez declined any medical attention and said that he felt fine. However, he had made a half-hearted attempt to hang his 300 lb. frame in the jail cell immediately after his arrest, and after speaking to police and prosecutors, he was sent to Bellevue Hospital for a psychiatric evaluation. His attorney filed a notice of psychiatric defense, an affirmative defense in New York, and sought to prove that disability through an expert. The doctors at Bellevue who initially examined Perez concluded that Perez had an antisocial character structure with a long-standing pattern of committing and even enjoying violent, sadistic acts, but that he gave no evidence during the interview of any symptoms consistent with dissociative identity disorder.

At suppression hearings held many months later, Perez claimed that the confessions were false. However, in the Drakeford confession, Perez had concocted a bizarre story of being swept up in a retaliatory killing by drug dealers, who enlisted his help in disposing of the body. In the Pollard confession, when he was talking with Detective Davis, Perez never said anything about hearing voices, a strange being called the “beast” telling him what to do, or the walls turning red. But, in his videotaped statement, Perez claimed that he heard voices and saw things, and that he went through emotional stages. He said he had an alter ego, a “consultant,” that spoke to him even before he formulated the plan with Williams and Walker to kill Pollard. In preparation for trial with his expert, Perez gave a detailed history of communicating with his alter-ego named “The Beast,” who he averred caused a red color to permeate Perez’s surroundings when present, and who urged him to kill Pollard.

There was ample evidence in the hospital records, both sides’ expert reports, and in the confessions themselves to meet the affirmative defense, and Bernard Perez was convicted of both killings and sentenced to life without the possibility of parole. His two accomplices in the Pollard murder were likewise convicted, one by plea and the other after a separate trial.

This work presents a case study of an improbable psychiatric defense of a serial killer.

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#### Homicide, Confession, Psychiatric Defense



### F24 The Evidentiary Standard for Authenticating Social Media Posts in Litigation

Stephanie Domitrovich, JD, PhD\*, Sixth Judicial District of PA, Erie, PA 16501

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the trial court’s authentication standards regarding expert testimony in relation to social media posts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing knowledge regarding authenticating social media posts.

Since social media and networking accounts can be faked or hacked, the Pennsylvania Superior Court three judge panel ruled in *Commonwealth v. Mangel*, a case of first impression in Pennsylvania, that social media posts cannot be authenticated in criminal cases unless prosecutors can present evidence of who actually authored said social media posts.<sup>1</sup>

The procedural and factual background follows. At a hearing on the State’s motion in *Limine*, the prosecution presented testimony of an Erie County Detective as a computer forensics expert. The trial court questioned the expert as to whether she could “testify to a reasonable degree of computer and scientific certainty” that the defendant had actually authored Facebook® posts and chat messages. The expert replied she could not so testify. The expert also testified she had not obtained an IP address for the Facebook® account in question. The trial court ultimately denied the motion in *Limine*.

On appeal, prosecutors argued trial court had applied a higher burden of proof than was required, but the Superior Court disagreed. Noting the issue of authenticating social media posts is one of first impression in Pennsylvania, the appeals court relied heavily on its own 2011 decision in *Commonwealth v. Koch*, which dealt with cellphone text messages.<sup>2</sup> The *Koch* court held that “authentication of electronic communications, like documents, requires more than mere confirmation that the number or address belonged to a particular person. Circumstantial evidence, which tends to corroborate the identity of the sender, is required.”

The PA Superior Court in *Mangel* pointed to various rulings by the United States Court of Appeals for the Second and Seventh Circuits, as well as state courts in Maryland, Massachusetts, Mississippi, and Texas, noting these courts required some evidence of authorship in order to authenticate social media posts. In *Mangel*, PA Superior Court stated the State “presented no evidence, direct or circumstantial, tending to substantiate that Defendant created the Facebook® account in question, authored the chat messages, or posted the photograph of bloody hands.” Moreover, prosecutors failed to produce any evidence that the defendant created or had access to the email accounts or phone number associated with the Facebook® account. Nor did prosecutors establish any relationship between the defendant and the owner of the phone number. In a separate footnote, the PA Superior Court noted the trial court correctly applied the expert testimonial standard when questioning the expert as to whether she could testify to a reasonable degree of certainty that the Facebook® posts were authored by the defendant. The PA Superior Court stated: “The mere fact that the Facebook® account in question bore Mangel’s name, hometown, and high school was insufficient to authenticate the online and mobile device chat messages as having been authored by Mangel.” The PA Superior Court further stated: “Moreover, there were no contextual clues in the chat messages that identified Mangel as the sender of the messages.” The prosecution also did not provide any evidence of the dates or times of the posts.

As emails, text messages, and social media data are increasingly used as common and powerful evidence at trial, new legal issues are being applied to the litigation process. Anyone collecting this evidence for use in criminal or civil litigation must authenticate and prove who owns the social media account, as well as provide at least circumstantial evidence of authorship of any specific posts, messages, or pictures. Direct testimony from the author admitting to having written the post or message in question is vital. Otherwise, parties wishing to use social media evidence must have circumstantial evidence as to authorship directly from social media companies, such as timestamps for messages, IP addresses for the devices making the posts, or other metadata.

#### Reference(s):

1. *Commonwealth v. Mangel*, 181 A.3d 1154, 1164 (Pa. Super. Ct. 2018).
2. *Commonwealth v. Koch*, 39 A.3d 996, 1005 (Pa. Super.2011).

#### Social Media, Evidence Admissibility, Authentication



### F25 Emerging Trends in Electronic Surveillance

Gary McDonald, Jr., JD\*, Dallas County Criminal District Attorney's Office, Dallas, TX 75063

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**Learning Overview:** The purpose of this presentation is to educate attendees regarding emerging and restrictive trends in electronic surveillance search and seizure law.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the limitations of personal expectations of privacy in public places as it relates to the usage of electronic devices or personal movements.

This presentation will inform attendees about recent trends in electronic surveillance law and restrictive trends in case law from the United States Supreme Court. This presentation will provide insight into the Court's interpretation of an individual's expectation of privacy in public locations and discuss long-standing techniques employed by law enforcement that are limited by the Court's holdings. These investigative techniques will include discussions of the usage of historical cell site location information and toll records, together with such investigative devices as pen registers and trap and trace devices.

On June 22, 2018, the United State Supreme Court handed down its opinion in *Carpenter v. United States*.<sup>1</sup> In *Carpenter*, the Court extended the protections of the Fourth Amendment to cell site location information in the possession of wireless service providers, requiring a search warrant issued upon a finding of probable cause for law enforcement to access such information. Prior to *Carpenter*, the United States Supreme Court handed down its opinion in *United States v. Jones*. In *Jones*, the Court likewise extended the protections of the Fourth Amendment to law enforcement's installation and use of mobile tracking devices by holding that such investigative techniques constitute a search. Together, these cases recognize increasingly greater expectations of privacy in public places than have been historically recognized by the Court.

Historically, information held in the possession of businesses, such as electronic service providers, excluding the content of communications, has not been protected by the Fourth Amendment's warrant requirement. Indicative of this is that federal statutory provisions, as well as the statutes of most states, do not require a search warrant to access most information. This presentation will analyze the shift in historical perspective that contributed to the privacy interests recognized in *Carpenter* and *Jones*.

While statutory authority may exist in state and federal jurisdictions allowing law enforcement to access electronic customer data on standards lower than required by *Carpenter* and *Jones*, the Court has severely limited law enforcement's access to certain types of information by requiring judicial approval on a finding of probable cause. This presentation will provide insight into those differences.

After attending this presentation, attendees will have a greater understanding of electronic surveillance tools in use by law enforcement and how those tools benefit investigations. Attendees will also have a greater understanding of emerging trends in courts providing increasingly greater protections to an individual's expectation of privacy in certain information, including the nature and extent of judicial process required for law enforcement to access such information.

**Reference(s):**

1. *Carpenter v. United States*, 585 U.S. \_\_\_\_ (2018).
2. *United States v. Jones*, 565 U.S. 400 (2012).

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**Privacy, Searches, Electronic Surveillance**



### F26 Expert Challenges Using Industry Standards

*John J. Lentini, BA\*, Scientific Fire Analysis, LLC, Islamorada, FL 33036*

**Learning Overview:** After attending this presentation, attendees will understand: (1) there are objective standards for the knowledge required of experts in many forensic disciplines, (2) too many practitioners who hold themselves out as experts do not meet the minimum criteria for professional qualifications, and (3) a qualifications challenge is more easily crafted and more likely to succeed than a methodological challenge.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that challenging an expert on methodological grounds is often more difficult than simply challenging the expert's qualifications.

Federal Rules of Evidence (FRE) Rule 702 (and most states' Rules of Evidence) states, "A witness who is qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise if: (a) the expert's scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or to determine a fact in issue; (b) the testimony is based on sufficient facts or data; (c) the testimony is the product of reliable principles and methods; and (d) the expert has reliably applied the principles and methods to the facts of the case."

Confronted with the possibility of challenging an expert opinion, most attorneys base their challenges on the four lettered requirements and are required to get into the weeds with an expert on the subject of methodology. Given the wide variety of forensic science disciplines, this places the lawyer in an almost impossible situation of arguing methodology with someone who is far more qualified to discuss methodology.

A strategy that is more likely to succeed is using the opening phrase of Rule 702 to challenge the qualifications of the expert. This does not require the attorney to go beyond learning what are the expectations of the discipline for training and knowledge. In some forensic disciplines, challenging qualifications will be difficult, but in others, it is the easiest means of persuading the court (or adverse counsel) that the expert will not be helpful to the trier of fact.

With respect to arson cases, unless an attorney specializes in insurance defense, he or she is likely to encounter one or two contested arson cases in a career. This necessitates consulting with an expert, but even a brief consultation will make the attorney aware that there exists an industry standard for the knowledge required to investigate fires.<sup>1</sup> Thus, the question becomes not "How did you reach your opinion?" but rather "Are you really qualified to express an opinion?" Per experience, the unfortunate answer to the second question is "No"—not if any kind of objective standard is consulted. A large cadre of supposedly professional fire investigators cannot explain the basic concepts of energy, power, heat flux, and fire pattern development. For example, there are many fire investigators who will be unable to tell you that a watt is 1 joule per second or explain the difference between energy and power or tell you the units used to measure radiant heat flux. When sponsoring counsel understands that their expert, who is likely to be the pivotal witness in a case, cannot demonstrate qualifications in even the most basic fire science, charges are likely to be dismissed or civil cases are likely to settle. No formal challenge is even necessary. If the court still allows the expert to testify, the jury will at least understand that they are hearing opinions from an unqualified individual.

This presentation will provide several examples of individuals who failed to demonstrate that they were even marginally qualified, and thus civil cases were settled or criminal cases were dismissed outright.

Several disciplines besides fire investigation (drugs, bloodstains, DNA, computer forensics) have adopted training guides or other documents that define the qualifications that experts should possess, and these can be used in a similar fashion.

#### **Reference(s):**

<sup>1</sup> NFPA (2014), NFPA 1033, *Standard for Professional Qualifications for Fire Investigator*, Quincy MA, National Fire Protection Association.

#### **Industry Standards, Qualifications, Challenge**



### F27 National Fire Protection Association (NFPA) 921 in Court: Lessons From 500 Case Decisions Over a Quarter Century and Across Two Countries

Terry-Dawn Hewitt, LLM\*, McKenna Hewitt, Denver, CO 80224-3703; Wayne J. McKenna, LLB, McKenna Hewitt, Denver, CO 80224-3703

**Learning Overview:** For attendees who are involved with fire cases, the purpose of this presentation is twofold: (1) to highlight the reasons why judges have cited NFPA 921 Guide for Fire and Explosion Investigations in court decisions, and (2) to predict future issues likely to be raised in civil and criminal cases by evaluating revisions in the latest edition of NFPA 921 taken together with the large body of existing cases. For those who are involved in forensic science disciplines other than fire investigations, the lessons from the use of NFPA 921 in court may shed light on how issues based on industry standards in other fields could develop once more members of the legal community become familiar with such standards.

**Impact on the Forensic Science Community:** It is rare to have an industry standard or guide published as recently as 1992, which has produced such a huge body of written decisions from courts in not one, but two countries. With the increasing emphasis on standardization and certification that the National Academy of Sciences/National Research Council (NAS/NRC) Report has inspired, it is incumbent on lawyers and forensic science experts to understand the many ways that industry standards can be used in court.<sup>1</sup> This presentation will impact the forensic science community involved in fire investigations by summarizing the lessons to be learned from the NFPA 921 cases. It will affect those in other disciplines by identifying the types of issues that they can expect to face when the many ways that industry standards can be used in civil and criminal cases becomes widespread knowledge among lawyers and judges.

The first edition of NFPA 921 was published in 1992.<sup>2</sup> A year later, the first mention of NFPA 921 was made by a judge in a written court decision. In the 25 years since, there have been more than 400 written case decisions specifically mentioning NFPA 921 in the United States, and another 60 in Canada. These numbers do not include the thousands of cases across both countries where NFPA 921 is mentioned by experts in their testimony or reports, nor where it is referred to by counsel in written briefs or oral argument. While it is common practice for trial lawyers and experts to use “learned treatises” (the legal term for authoritative publications) in various ways, it is very rare for so many state, provincial, federal, and appellate courts in two countries to expressly mention an authoritative publication in reasons for their decisions.

This presentation will examine the past lessons and the future trends in fire investigations that can be learned from these court decisions. It includes a timeline analysis showing the growing influence of NFPA 921. A geographical breakdown is also incorporated so attendees can consider whether the use of NFPA 921 in court is truly an international phenomenon or simply concentrated in localized jurisdictions. The most significant issues that the courts have considered are delineated, as well as some of the litigation effects, such as disqualification of experts and post-conviction relief in criminal cases. The latest edition of NFPA 921 is then considered in the context of the existing case decisions to predict future issues in fire litigation.<sup>1</sup> Finally, lessons learned from NFPA 921 in court are generalized to provide insights for those in other forensic science disciplines as to ways that their industry standards and guides may be used in civil or criminal litigation.

#### Reference(s):

- <sup>1</sup> National Research Council (NRC), Committee on Identifying the Needs of the Forensic Science Community. (2009). *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press.
- <sup>2</sup> Tech. Committee on Fire Investigations. *NFPA 921 Guide for Fire and Explosion Investigations*. (Quincy, MA: Natl Fire Protection Assn, 1992 to 2016.)

#### Fire Investigation, NFPA 921, Consensus Standards



### F28 Three Attorneys Participate in Fingerprint Proficiency Testing: Results and Observations

*Brendan P. Max, JD\**, Cook County Public Defender, Chicago, IL 60602; *Joseph Cavise, JD\**, Cook County Public Defender, Chicago, IL 60602; *Richard E. Gutierrez, JD\**, Law Office of the Cook County Public Defender, Chicago, IL 60602

**Learning Overview:** The goal of this presentation is to generate discussion regarding the level of competency assessed by current fingerprint proficiency tests and the limitations of inferences to be drawn from successful participation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by generating robust discussion of, and possibly reform to, current fingerprint proficiency testing practices.

Proficiency testing has long played a very important role in the forensic science community in general, and the forensic fingerprint community in particular. For years, fingerprint practitioners have relied on fingerprint proficiency testing results when making claims about the overall reliability of the Analysis, Comparison, Evaluation-Verification (ACE-V) method when making assertions about the reliability of particular forensic laboratories and when making assertions regarding the level of competency of individual fingerprint examiners. Some within the fingerprint community have pointed to extremely high passage rates when asserting that the overall error rate in the discipline is low. Separately, laboratories routinely include participation in proficiency testing as a major component of quality assurance programs. Forensic laboratories point to near-universal passage of fingerprint proficiency tests as an important indicator of a lack of quality assurance problems within laboratories, and as an indicator that substantial retraining of fingerprint examiners is not necessary. Finally, individual forensic fingerprint examiners routinely testify during expert *voir dire* to their participation in annual fingerprint proficiency testing.

Especially in the case of uncertified fingerprint examiners, results of fingerprint proficiency testing are often the only objective evidence offered as a means to establish the competency required to be admitted for testifying as an expert in a criminal trial. In this regard, fingerprint examiners regularly tout error-free passage of fingerprint proficiency testing as an important signal to the criminal justice system that they can competently conduct forensic fingerprint examinations and reliably associate a latent print with a suspected source. However, each of these assertions that are based on participation in fingerprint proficiency testing—low overall error rates, lack of quality assurance problems, and individual examiner competency—are only supported in a meaningful way if the fingerprint proficiency tests are rigorous enough. Fingerprint proficiency tests that are too easy do not provide an accurate indication of overall error rates in the field.<sup>1</sup> Similarly, easy fingerprint proficiency tests do not meaningfully advance quality assurance programs, as they are not designed to detect important breaking points within a laboratory and across the discipline, and therefore provide only minimal guidance for retraining of examiners. Finally, successful participation in easy proficiency testing by individual fingerprint examiners should not indicate competency in any but the easiest comparisons encountered in casework.<sup>2</sup> Given the critical question of whether current fingerprint proficiency tests are easy or rigorous, this study sought references in the scientific literature to answer this important question. Finding very limited discussion of fingerprint proficiency testing by practitioners in the discipline, this study sought to investigate this question in one modest way—through participation in 2018 fingerprint proficiency testing.

This presentation will include discussion of the results of participation in fingerprint proficiency testing (a combined 33 consensus responses out of 36 fingerprint comparisons with no false positive errors). Through further discussion of the study's participants' obvious lack of formal training in forensic fingerprint comparisons, as well as a detailed discussion of the test itself, it will be suggested that the current fingerprint proficiency testing regime is not rigorous and needs to be reformed.

#### Reference(s):

- <sup>1</sup> William Thompson, John Black, Anil Jain, and Joseph Kadane. *Forensic Science Assessments: A Quality and Gap Analysis-Latent Fingerprint Examination*. AAAS (September 2017).
- <sup>2</sup> Koehler, J.J., Forensics or Fauxrensic? Ascertaining Accuracy in the Forensic Sciences. *Arizona State Law Journal*, 49, 1369-1416 (2017).

#### Fingerprints, Proficiency Testing, Competency



### F29 The Regulation of Forensic Science Evidence in Europe

*Joelle V. Vuille, PhD\*, University of Lausanne, Lausanne 1015, SWITZERLAND*

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**Learning Overview:** The goal of this presentation is to discuss the current state of regulation in forensic science in various European countries, highlighting the benefits and challenges that current regulation regimes pose in terms of a fair administration of justice and some of the regulatory trends that can be expected in the future.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering an alternative perspective on forensic science regulation by discussing the options taken by European countries on the matter in various forensic disciplines and the challenges that such regulatory schemes raise with regard to the right to a fair trial.

The ability of forensic practitioners to provide the fact-finder with accurate information has been questioned in the past decade. In the United States, several forensic disciplines have been denounced as lacking empirical validation, relying too heavily on the subjective opinion of individual experts, and having inconsistent and sometimes shoddy practices. Institutions have thus been tasked with harmonizing existing regulation, reforming outdated rules, and developing new standards when needed. In turn, these efforts have given rise to vigorous debates in the forensic and legal communities.

In Europe, each country regulates the production and use of forensic science evidence in their own criminal proceedings. Very few efforts have been made to develop a coherent system of regulation, although the growing number of transnational prosecutions and the increase in exchange of evidence between countries would require some form of harmonization. Besides, it could be argued that some practices run counter to the right to a fair trial as guaranteed by the European Convention on Human Rights because they do not give the parties an opportunity to substantively assess the robustness of the expert conclusions presented in a given case.

Using practical examples, this presentation will show how forensic science evidence is currently regulated in the criminal justice systems of Europe. It will highlight the high degree of variability in regulation between different countries and disciplines, and its consequences in terms of the administration of justice. Finally, it will illustrate what dangers such unsystematic regulatory regimes pose in terms of the rights of the parties (notably, the defense). Finally, the presentation will offer an evaluation of the relevance of current regulatory regimes considering existing knowledge in the fields of forensic science, law, and governance.

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#### Regulation, Standards, Europe





### F30 The International Criminal Court and the Forensic Science Community

Victor W. Weedn, MD, JD\*, The George Washington University, Washington, DC 20007

**Learning Overview:** After attending this presentation, attendees will be familiar with the International Criminal Court and understand the role of the forensic science community in matters of the court.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by familiarizing attendees with the court and the role of forensic science in court prosecutions.

**Brief Synopsis:** International criminal prosecutions of the International Criminal Court (ICC) use forensic evidence. A Scientific Advisory Board (SAB) of the Office of The Prosecutor (OTP) oversees this evidence. It is thought by some that the Academy should participate in this board.

The ICC is the product of the Rome Statute, which was adopted in 1998 and went into force in 2002.<sup>1</sup> The ICC is located in the Hague, Netherlands. The ICC follows in the tradition of the Nuremberg trials, and the International Criminal Tribunal for the former Yugoslavia (ICTY) and the International Criminal Tribunal for Rwanda (ICTR) tribunals. Unlike these *ad hoc* predecessors, the ICC is a permanent standing fixture.<sup>2</sup>

The ICC is an international organization (IO), independent from, but associated with, the United Nations (UN). The International Court of Justice (ICJ) is the primary judicial organ of the international community for settling civil disputes between nation-states, while the ICC prosecutes criminal cases.

Through prosecution of crimes against humanity, the hope is that the ICC will be an instrument of world peace. It is the court of last resort for the most serious crimes of concern to the international community. Specifically, the court has jurisdiction over genocide, crimes against humanity, war crimes, and crimes of aggression. The Statute allows the ICC to prosecute crimes committed on the territory of a court member or by a national of a court member. A member state may refer a situation, the UN Security Council may do so, and the prosecutor may, under the so-called *proprio motu* power, initiate an investigation. The ICC accepts cases when national judicial systems are unable or unwilling to prosecute. The ICC will not prosecute persons under the age of 18 years.

The ICC is governed by the *Assembly of State Parties*, which consists of representatives of the current 123-member states. Many of the world's major powers are not members, citing national sovereignty concerns. The ICC consists of four organs: The Presidency, The Chambers, The Office of the Prosecutor, and The Registry. The ICC is dependent upon cooperation of nations to do its work; it has no police force of its own.

The ICC has had to establish its own criminal law, but to date does not yet have a well-developed and robust body of evidence law. Forensic evidence is used in the prosecutions. A Forensic Science Section was created in the Office of the Prosecutor. Dr. Eric Baccard was the first head of the Section. Forensic pathology and forensic anthropology were the primary scientific evidentiary disciplines, but now digital evidence and satellite imagery play a larger role. The American Association for the Advancement of Science (AAAS) has recently published recommendations with regard to geospatial evidence.<sup>3</sup>

The creation of a Scientific Advisory Board (SAB) was recommended by Dr. Baccard in 2005 and 2007, and then was incorporated into the recommendations of the Human Rights Center of the UC Berkeley Law School in 2012. A planning meeting was held in 2013. The inaugural meeting of the SAB was held in 2014. Dr. Duarte Nuno Vиейro was elected the first SAB Chair. The SAB is composed of the Presidents, or their designees, of 18 international forensic and legal medicine associations. The SAB membership does not include the American Academy of Forensic Sciences (AAFS); as the AAFS is the largest international forensic science organization, this seems unfortunate.

#### Reference(s):

1. International Criminal Court website: <https://www.icc-cpi.int/Pages/Main.aspx>.
2. AAAS website: *Geospatial Evidence in International Human Rights Litigation: Technical and Legal Considerations*, <https://www.aaas.org/report/geospatial-evidence-international-human-rights-litigation>.
3. Bosco D. *Rough Justice: The International Criminal Court in a World of Power Politics*. Oxford University Press, NY, NY, 2014.

#### International Criminal Court, Human Rights, Crimes Against Humanity



### **F31 False Positive Breath Alcohol After Pharyngeal Spray Treatment**

*Duygu Yavuz\*, Uskudar University, Istanbul 34664, TURKEY; Tugba Ünsal, PhD, Üsküdar University Department of Forensic Science, Istanbul 34662, TURKEY; Sevil Atasoy, PhD, Uskudar Universitesi Adli Bilimler Enstitüsü, Istanbul, TURKEY*

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**THIS ABSTRACT WAS NOT PRESENTED.**

### F32 Self-Determination and Living Wills: The Actual Italian Context After the Promulgation of the 2017 Law 219 on End of Life Issues

*Matteo Scopetti, MD, Sapienza University of Rome, Rome, 00161 ITALY; Vittorio Gatto, MD, Sapienza University of Rome, Rome, 00161 ITALY; Alessandro Santurro, MD, Sapienza University of Rome, Rome, 00161 ITALY; Mariantonio Di Sanzo, MD\*, Rome, ITALY; Raffaele La Russa, MD, Rome, Lazio, ITALY; Paola Frati, PhD, Department of Anat Histol Forensic and Orthop Sciences, Roma 00161, ITALY*

**Learning Overview:** After attending this presentation, attendees will better understand how informed consent and living wills are actually regulated in Italy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing the innovative principles and the provided solutions to an issue not yet explicitly regulated in the Italian context.

The Italian Parliament has recently approved the Law 219/2017 regarding “Rules on informed consent and advance provisions of treatment.” This presentation aims to briefly outline the key points of the Law in order to provide meaningful insights on the end of life issues for health care stakeholders. The provision has been analyzed in consideration of Italian deontological, juridical, and jurisprudential context as well as the statements of the “Convention on Human Rights and Biomedicine.”

The current legislation addresses the sensitive issue of informed consent, regulating the modalities of expression and revocation, as well as the advance provisions of treatment, in order to allow the individual to declare his or her orientation on the end of life, in the event of an impossibility to express one’s own will.

The normative text of Law 219/2017 is composed of eight articles. The first article is dedicated to informed consent, with particular attention to the relationship of trust between doctor and patient, with the involvement of the family members of the latter, the cohabitant, or other trusted persons. The same article regulates the right to information of every person to know their health conditions and therefore, to be informed in a comprehensible manner in relation to the diagnosis, prognosis, risks, and benefits of health treatment and the consequences for the case of rejection of the treatment itself. Informed consent can be expressed in writing and must be included in the medical record, even if it is possible to express this consent by videotaping or other electronic communication equipment. Informed consent can always be revoked, and the principle is established that every person of age, obviously capable of understanding and wanting, has the right to refuse any medical treatment. In cases of emergency situations, the doctor is always obliged to ensure essential health care to the patient, respecting, if possible, the will of the same. According to the provisions of the third article, informed consent from minors is expressed or refused by parental responsibility. Consent may also be expressed or refused by the support administrator, where appointed, considering the will of the beneficiary.

The second article is dedicated to the treatment of pain or the prohibition of unreasonable obstinacy in treatment in the final phase of the patient’s life. In particular, the physician is obligated to work to alleviate the patient’s suffering, even in cases of refusal of consent to health treatment, through the provision of palliative care.

Article 4 deals with the advance provisions of treatment, acts in which any adult person who is capable of understanding and is willing, can express their preferences and convictions regarding health care, being able to also appoint, by written deed, a fiduciary subject that represents him/her in relations with the doctor and the hospitals.

In the fifth article, the Law provides for the possibility of defining a shared care plan between doctor and patient, to which the health professional is obliged to comply if the patient is unable to give his consent.

In view of the stated principles, the Law represents a crucial novelty, dealing with relevant issues that have been the subject of contradictory judgments ruled upon by the Italian courts. In fact, the provision guarantees the patient’s right to self-determination, dignity, and well-being through the regulation of the relationship between patient and doctor. Although the law states essential principles, some inconsistencies remain, especially regarding the issue of minors. For this reason, it is necessary to continue to deepen the collaboration between health professionals, bioethicists, and jurists in order to translate the new rules into practice.

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#### Self-Determination, Living Wills, End of Life



### F33 Depleted Uranium and Cancer Risk: A Case Report

*Martina Fichera, MD\**, University of Catania, Catania 95123, ITALY; *Giuseppe Davide Albano, MD*, Foggia 71121, ITALY; *Aldo Liberto, MD\**, University of Catania, Catania 95123, ITALY; *Francesco Amico, MD*, Medicina Legale, Catania 95123, ITALY; *Maria Tea Teodoro, MD*, Catania 95123, ITALY; *Massimiliano Esposito, MD\**, University of Catania, Catania 95123, ITALY; *Diego Geraci, PhD*, Catania 95100, ITALY; *Orazio Cascio, MD*, University of Catania, Catania, ITALY

**Learning Overview:** The goal of this presentation is to examine the effects of uranium exposure on human health and disease. In this presentation, the case of an Italian soldier who has contracted brain cancer is discussed. The case of human uranium exposure during military missions came to the attention of the judicial authorities during arranged investigations of inspection.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the relationship between environmental pollution by depleted uranium and the possible effects on cancer.

The purpose of this work is to analyze the relationship between environmental pollution from depleted uranium and possible health effects. Depleted Uranium (DU) is generally considered an emerging pollutant and is a known carcinogen. DU has been hypothesized to represent a hazardous element both for exposed soldiers and the inhabitants of polluted areas in the war zones. In Afghanistan and Iraq, it was shown that the illnesses and deaths of military personnel were related to environmental pollution caused by the explosion of depleted uranium munitions. The harmful effects on personnel exposed without protection are known, especially in the famous “Gulf syndrome” that affects many United States military directly (with oncological diseases) and indirectly (malformations of the children of soldiers returning from Iraq). These diseases were then recognized by the American government and by the military and these families were compensated. Also, the Balkan region suffers from DU pollution in many areas and the effects of this can damage public health through poisoning and the increase in incidence of various cancers. The overall incidence of lung cancer, leukemia, and lymphoma has increased in Italian soldiers exposed and several judgments of the Court of Cassation have recognized the causal link between exposure to DU and the development of tumors, condemning the Ministry of Defense to pay damages.

Unlike the United States military, Italian soldiers on mission in these territories did not have the necessary protective devices and Italian military personnel were in contact with DU. The equipment provided for the military personnel was not adequate and did not meet the standards established for the territories with possible contamination by Nuclear, Biological and Chemical (NBC); the army has been exposed to significant risk factors (i.e., air pollution, toxic contamination, exhaust fumes, and chemical solvents used to clean weapons) that would contribute to cancer development.

**Case Report:** An Italian navy sergeant who was exposed to DU during missions in Kosovo, Yugoslavia, and Albania from 1999 to 2001 and who has no cancer or anamnesis for cancer. The military has been exposed to carcinogenic risk factors, in particular the toxic contamination caused by the dispersion of heavy minerals in the environment produced by the explosion of DU munitions and the contamination of water and air in the workplace. In 2006, after the onset of neurological symptoms (convulsions), the Italian soldier was admitted to the hospital and was diagnosed with a brain tumor—oligoastrocytoma. The tumor was treated surgically and with radiation, yet eventually caused the sergeant’s death in 2013.

Although oligoastrocytoma does not belong to tumors more frequently related to depleted uranium exposure, exposure to this substance is highly likely to have led to the onset of cancer and subsequent death.

#### Oligoastrocytoma, Depleted Uranium, Cancer Risk



### F34 Should Forensic Science Rely on Opinions or Factual Data? The Role of Forensic Metrology

Alessandro M. Ferrero, MSc, Politecnico di Milano, DEIB, Milano 20133, ITALY; Veronica Scotti, LL.M\*, Politecnico Di Milano, DEIB, Milano 20133, ITALY

**Learning Overview:** After attending this presentation, attendees will better understand the role played by forensic metrology in changing an opinion, including competent opinions based on scientific data, into a set of factual data within which the information we are looking for lies with a given probability.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the manner in which expert testimonies aimed at reporting on experimental tests (i.e., fingerprint comparisons, DNA comparisons, exceeded thresholds, etc.) become more complete and informative if reported in a metrologically sound way, including the evaluation of measurement uncertainty.

Despite the emphasis given by the 2009 National Academy of Sciences (NAS) Report and the 2016 President's Council of Advisors on Science and Technology (PCAST) report to the need for scientifically validated forensic methods, it is still common to refer to forensic-science *opinions*, even when the expert witnesses are reporting the results of experimental tests or activities.<sup>1-3</sup>

The PCAST report clearly emphasizes the demand that the scientific validity of forensic science methods is assessed before these same methods can be considered in courtrooms.<sup>2</sup> In other words, forensic methods should provide scientifically validated data, not opinions. The report also provides indications on how to assess this validity through the definition of the *foundational validity* and the *validity as applied*, but unfortunately, it fails to show how such terms can be quantitatively evaluated.

Probability is generally considered to provide such a quantification or, at least, through the evaluation of suitable *likelihood ratios*, provide a quantitative way to assess which one, between two hypotheses, is the most probable.<sup>3</sup>

The major drawback is that probability may not provide an undoubtable answer in all specific situations (Does this fingerprint belong to the defendant? Does this DNA pattern belong to the suspect?), since it is generally evaluated on available databases and does not consider the employed instruments, the influence quantities, the operator, and other variables.

This presentation seeks to show how metrology can overcome this potential drawback, since the good measurement practice does consider all possible contributions to uncertainty, from the *definitional* contribution that quantifies the foundational validity to the many instrumental contributions that quantify the validity as applied.<sup>4</sup>

The result is still a probability, or better, a standard deviation (standard uncertainty) that yields an interval of possible values, built about the measured value, within which the unknown true value of the quantity subject to measurement is supposed to lie with a given coverage probability.<sup>5</sup>

It will also be shown how, starting from such a coverage interval, it is still possible to evaluate a likelihood ratio, if needed, which is now based on specific data related to the considered measurement or test and is supposed to be more reliable in helping the trier of fact render a fair decision beyond any reasonable doubt.

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4. Ferrero, Alessandro and Scotti, Veronica. An Interpretation of the 2016 PCAST Document in Terms of Forensic Metrology. *Proceedings of the American Academy of Forensic Sciences*, 70<sup>th</sup> Annual Scientific Meeting, Seattle, WA. 2018. 581.
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#### Opinions, Data, Uncertainty



### F35 A Metrologically Sound Assessment of Blood Alcohol Concentration (BAC) Through a Breath Alcohol Concentration (BrAC) Measurement

Alessandro M. Ferrero, MSc, Politecnico di Milano, DEIB, Milano 20133, ITALY; Veronica Scotti, LLM\*, Politecnico Di Milano, DEIB, Milano 20133, ITALY

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the different sources of uncertainty that affect BAC measurements when performed through a BrAC measurement, especially when the major source of inaccuracy does not come from the employed instrument but is implied in the way BrAC is reported to BAC.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a metrologically sound approach to BAC measurement and by understanding its uncertainty when it is obtained through a roadside BrAC measurement. The multiplicative coefficient to get BAC from BrAC is discussed and how its foundational validity can be stated will be shown, beginning with the available literature and quantified in terms of definitional uncertainty.

Roadside BrAC tests have become the standard method to assess BAC in drivers and represent the most effective tool to fight against DUI crimes in most developed countries. On the other hand, the validity of the obtained measurement results is often challenged and the employed BrAC instruments are considered the major cause of inaccuracy.<sup>1,2</sup>

However, from a strict metrological perspective, this contribution to measurement uncertainty only represents the instrumental uncertainty contribution and yields a quantification of the *validity as applied*, as defined by the President's Council of Advisors on Science and Technology (PCAST) report.<sup>3,4</sup>

Another important contribution to uncertainty, the definitional uncertainty contribution, that quantifies the *foundational validity*, as defined by the PCAST report, has not been considered nor quantified. As a matter of fact, the choice of the proportionality factor to convert the measured BrAC into a BAC, is critical and represents the major source of uncertainty of this method.<sup>3,4</sup>

This presentation seeks to show how this contribution can be quantified, beginning with the data available in the scientific literature combined with the instrumental uncertainty to evaluate the overall uncertainty in the determination of a BAC starting from a BrAC measurement.<sup>5,6</sup>

This presentation will also show how, starting from the obtained uncertainty value, it is possible to evaluate the probability that a BAC value higher than a given threshold may actually be lower than the threshold, thus quantifying the doubt of not making a right and fair decision.

#### Reference(s):

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3. President's Council of Advisors on Science and Technology (PCAST). *Report to the President: Forensic Science in Criminal Courts: Ensuring Scientific Validity Of Feature-Comparison Methods*. (2016). Washington, DC: Executive Office of the President of the United States.
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#### BrAC, BAC, Uncertainty



### F36 Preferred Crime Scene Documentation Methods

*Giovanna M. Vidoli, PhD\**, University of Tennessee, Knoxville, TN 37996; *Michael W. Kenyhercz, PhD*, Department of Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickam, HI 96816; *Joanne Devlin, PhD*, University of Tennessee Department of Anthropology, Knoxville, TN 37996; *Jason J. Keller, MFS, NCIS*, Great Lakes, IL 60088

**Learning Overview:** The goal of this presentation is to provide the forensic science community with data regarding the monetary, temporal, and interpretative advantages or disadvantages of utilizing 3D laser scanners in scene documentation as compared to traditional documentation methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data on how 3D scanned products perform compared to other documentation methods and how much novel data can actually be gleaned from the scans as compared to traditional mapping methods.

Technological advances have allowed for a decrease in the costs associated with 3D laser scans and an increase in the availability of scanners for crime scene investigators. However, the value of this new technology in crime scene documentation as compared to traditional techniques (i.e., hand-drawn maps, photographs) is largely unknown. This presentation will provide the scientific and medicolegal community with quantitative data on a layperson's and a forensic professional's interpretation and assessment of traditional scene documentation and 3D laser-scanned representations of potential crime scenes. This presentation's proposition is that 3D laser-scanned images do not always provide the best information to a jury.

A total of four indoor and eight outdoor mock crime scenes involving human remains, or a proxy, were documented using a 3D laser scanner and multiple traditional means, including photographs, hand-drawn and total station/GIS maps, and detailed notes. The outdoor scenes included human skeletal or mummified remains on the ground surface and in burials established at the Anthropology Research Facility at the University of Tennessee, Knoxville. Following documentation, 200 people were surveyed and asked to review and compare the documentation products. Respondents were required to rank the method that best represents a given crime scene scenario with respect to human remains and associated evidence. As a result, there is quantifiable data regarding the effectiveness of 3D laser-scanned images as compared to traditional documentation images. In addition, this study conducted cost/benefit analysis for all documentation methods, noting the time and expenses (personnel and equipment) associated with each method. This will provide agencies with data in making training and budgetary decisions regarding the acquisition of such instrumentation.

The survey results indicate that while many participants appreciate the detail and clarity provided by 3D scanning, the use of such may not always be the most effective and/or efficient use of an agency's resources. For the current study, examination of the age cohort on respondent answers was analyzed. The age cohorts were grouped by 18–22 years ( $n=5$ ), 23–30 years ( $n=12$ ), 31–45 years ( $n=32$ ), 46–60 years ( $n=25$ ), and 61+ years ( $n=51$ ). Evidence preference was analyzed with a Kruskal-Wallis test in R. Results indicate that respondent age cohort does not impact the preference on site recording media. Overall, there is a preference for 3D renderings, with 80.2% preferring this media for site presentation. Photographs were the second most preferred method, with a 14.9% preference, followed by hand-drawn maps (3.3%), then 2D maps (1.6%). The 18–22 year cohort had a 100% preference for 3D renderings; the 23-30 year cohort was more varied with a 66.7% preference for 3D renderings, 12.7% for 2D maps, and 8.3% for hand-drawn maps and photographs, respectively. The 31-40 year age cohort had a 70.9% preference for 3D renderings, 22.6% for photographs, and 6.5% for hand-drawn maps. The 41-60 year cohort had a 96% preference for 3D renderings and 4% for hand-drawn maps. Last, the 61+ year cohort had a 79.6% preference for 3D renderings and 20.4% for photographs.

#### 3D Scanning, *Voir Dire*, Crime Scene Documentation



### F37 Developing an Introductory Analytical Science Training Program for Lawyers and Judges

Nicolas R. Hughes, JD\*, Harris County Public Defender's Office, Houston, TX 77002; Ashraf Mozayani, PharmD, PhD, Texas Southern University, Houston, TX 77004; Jasmine M. Drake, PhD, Texas Southern University, Houston, TX 77004

**Learning Overview:** After attending this presentation, attendees will better understand the role of analytical science in criminal cases and how to educate lawyers and judges regarding analytical evidence.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing an easily replicated model for a basic analytical science training program to improve the competency of lawyers and judges who encounter analytical evidence.

Scientific evidence is often the most persuasive evidence in a case. The 2009 National Academy of Sciences poignantly discussed the legal profession's insufficient understanding of scientific evidence.<sup>1</sup> A lawyer must be able to competently challenge scientific evidence to satisfy the Constitutional right to counsel.<sup>2</sup> A lawyer cannot ethically represent a client unless the lawyer is able to effectively challenge scientific evidence.<sup>3</sup> Failure to effectively challenge scientific evidence can result in wrongful conviction.<sup>4</sup>

Advances in analytical science have increased the sophistication of and reliance upon analytical evidence in the courtroom. Gas Chromatography/Mass Spectrometry (GC/MS) is used by many forensic disciplines to confirm the identity of unknown substances. DNA analysis is often used to connect evidence to a person. Analytical evidence is commonly encountered in many types of criminal cases, including prosecutions involving drugs, Driving While Intoxicated (DWI), and sexual assault. A metropolitan laboratory may annually analyze thousands of pieces of evidence affecting thousands of criminal cases.

While existing programs offer lawyers analytical science training, these programs are too expensive and too small to reach the entire legal community.<sup>5</sup> Existing programs are like a bespoke suit—high-quality and tailored for a specific fit—but the majority of people are better served by an affordable, nice suit from the rack of a department store. The Forensic Science Learning Lab, Harris County Public Defender's Office, and the National Association of Criminal Defense Lawyers partnered ("the Partnership") to create a model for an adaptable, expandable, cheaper alternative to other analytical science training programs while providing high-quality education. Similar programs can be offered by a wide range of qualified professionals nationwide. Programs can use existing educational facilities and funding resources to provide cheap or free training to lawyers and judges.

A basic analytical science course addresses the theory and knowledge needed to understand analytical evidence. The Partnership's model for a basic GC/MS training program includes the principles of GC/MS, methodology, documentation, data analysis, instrument design and maintenance, sample preparation, and demonstrations. The model features legal topics including the aftermath of the 2009 National Academy of Science Report, discovery, expert witnesses, and *Daubert/Frye* challenges.<sup>1</sup> Programs are tailored by jurisdiction and can include in-laboratory exercises and interactive problems. The Partnership is currently creating a lecture-based DNA program using the same general model used to create the GC/MS program.

Lawyers and judges need training regarding scientific evidence. Analytical science is commonly encountered in criminal cases and can be critical evidence. Basic analytical science training programs play an important role in the effective representation of the accused and in ensuring fair trials. The Partnership's model for an introductory analytical science training program is an adaptable, expandable, cheaper alternative to other programs and can meet the legal community's need for better scientific training.

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2. *Hinton v. Alabama*, 571 U.S. 263, 273 (2014).
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#### Continuing Education, Program Development, Analytical Science





### **F38 To Disclose or Not To Disclose: How Outcome-Based Case Theory Guides Information Gathering in Mental Health Evaluations**

*Jason D. Ricke, JD, LLM\*, Office of the Public Defender, Upper Marlboro, MD 20772*

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**Learning Overview:** After attending this presentation, attendees will understand how trial attorneys have conflicting views about whether to gather certain types of mental health data related to a defendant and whether unhelpful information is required to be disclosed to an expert witness working on the case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that expert witnesses rely on attorneys and their teams to provide comprehensive and accurate information about defendants when conducting mental health evaluations, and how attorneys may intentionally fail to acquire certain records or fail to disclose to their expert records that they deem unhelpful to the ultimate outcome they are seeking. The quality of a forensic expert's opinion is limited by the quality of information that forms the basis of that opinion. When an expert's conclusion is based on incomplete or inaccurate information, their opinion is subject to exclusion or attack on cross-examination.

Court-ordered mental health evaluations are legally significant in the context of competency to stand trial and criminal responsibility. Typically, when a court orders an evaluation, the attorneys from the government or the defense are responsible for acquiring information or mental health records to be used by the expert in forming their opinion. Attorneys may strategically choose to sacrifice a comprehensive record in order to obtain their desired result.

**Do They Have Time?** In state court prosecutions for cases not considered high profile, judges and attorneys are under constant pressure to resolve cases quickly and without unnecessary delay. Even if an assertion of incompetency to stand trial or lack of criminal responsibility is alleged, the court pressures the parties to resolve the case. The government and the defense may temper their efforts to obtain records in the name of efficiency, especially if they know those results may be harmful to the outcome they desire.

**Do They Have To Worry About the Opposing Party?** Attorneys on both sides of a mental health evaluation consider not only the forensic evaluator, but also opposing counsel and the assigned judge. If one party does not believe the other side will put forth efforts to uncover the missing or incomplete information, a calculated decision to purposefully withhold certain records could still produce the desired result. A detailed attorney thoroughly working up the case poses more of a threat to the unprepared expert.

**Do They Have to Disclose the Records?** Ethical rules for attorneys do not directly address disclosure of records to an expert witness, especially when the disclosure of those records is harmful to the interests of a defendant. While the prosecution may have a heightened responsibility to seek justice, if either side could access harmful information, a defense attorney may be acting against the interests of the defendant by providing certain information to an expert witness.

Mental health evaluations pose a unique challenge in seeking the truth because the information used to base those opinions is not consistent from case to case. As long as attorneys remain the gatekeepers to what information is given to and used by mental health evaluators, attorneys will continue to shape the results of those evaluations to each side's respective theory.

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#### **Mental Health, Disclosure, Attorney Ethics**



### F39 The Impact of Forensic Evidence on Drug-Related Offense Sentencing Outcomes

*Jennifer W. Bourgeois, MS\*, Texas Southern University, Houston, TX 77004; Jasmine M. Drake, PhD\*, Texas Southern University, Houston, TX 77004*

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**Learning Overview:** After attending this presentation, attendees will understand the intersection of forensic science and criminal justice case processing and the implications for policies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying trends in criminal justice case processing associated with drug-related offenses and identifying sources for backlogs by pinpointing specific reasons for untested forensic evidence and its influence on the cases of drug-related offenses.

This report will use Harris County District Clerk Court records and other available data to assess the relationship between untested forensic drug evidence and case dispositions. Recently, the Houston Forensic Science Center has taken steps to clear a backlog problem in its laboratory. The crime laboratory is not immune to recurring backlog problems or other struggles. The plan will span a period of ten months and would require \$2 million dollars to outsource DNA testing for approximately 1,000 cases. DNA sections within a crime laboratory are not the only divisions impacted with untested evidence. Toxicology and controlled substances sections can have long turnaround times, up to several months.

Regardless of the growth of forensic science services, published research related to the impact of forensic evidence and predictors of sentencing dispositions for drug-related offenses is lacking. The results of the first nationwide survey on untested forensic evidence was published in 2009. There are various reasons why evidence may not be tested, but there is a gap in the literature that discusses the impact on defendants and sentencing outcomes. Specifically, a question that is often not discussed is the relationship between untested evidence and the likelihood of someone accepting a plea deal. As a result of backlogs in crime laboratories, the criminal justice system can expect to see individuals make decisions that will have lasting consequences on their lives without the analysis of forensic evidence. Improper procedures and evidence tampering have troubled many crime laboratories across the nation, which has impacted past convictions and several thousand additional criminal court cases. Lengthy backloads have lasting concerns in each step in the criminal justice system process. Prior literature indicates inconsistencies in the relationship between forensic evidence and sentencing. Given the limited research on backlogs and its consequences on the front end of the criminal justice system, there is urgency in needing prompt action for reforming both forensic science and criminal justice practices.

Crime laboratories must work closely with law enforcement and the court system to ensure fair due process of law. Therefore, this report contributes a guide to identify sources for backlogs in a local crime laboratory by pinpointing specific reasons for untested forensic evidence and its influence on the case processing of drug-related offenses.

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#### Drug Evidence, Criminal Justice, Sentencing Outcomes



### F40 Problems and Possibilities With Foreign DNA in a Capital Post-Conviction Defense Investigation

*Matilde J. Carbia, JD\*, New Orleans, LA 70112; Kevin Riach, JD\*, Fredrikson & Byron, PA, Minneapolis, MN 55402*

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**Learning Overview:** After attending this presentation, attendees will understand creative solutions to the problems inherent in developing foreign DNA evidence in post-conviction, including: (1) resistance by the state and the courts to further development of old forensic evidence, (2) the need to use outdated or obsolete technologies to process newly collected forensic evidence to compare profiles developed in decades-old crimes, (3) the limitations of current technologies to process degraded forensic evidence, and (4) the practical challenges of incorporating newly processed and newly discovered forensic evidence in a post-conviction case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping attendees gain insight into the obstacles defense counsel faces when confronted with cold case DNA evidence and limited avenues for developing new forensic information, as well as practical solutions to these problems.

This presentation recently used the DNA profile developed from blood found at the scene of a 20-year-old quadruple homicide to connect an alternative suspect to the crime. In 1996, the state developed a profile from blood found on a red jacket left hanging at the crime scene that did not match the victims or the defendant. The profile was developed using the now-outdated DQ Alpha and Polymarker DNA technologies. Post-conviction counsel filed a motion for additional testing, based in part on this previously unidentified sample, which was opposed by the state and denied by the court. Years later, post-conviction counsel obtained DNA from an alternate suspect and located a lab that could still perform DQ Alpha and Polymarker DNA testing. That lab developed a profile from the newly collected sample that is the same as the previously unknown profile on the red jacket. This evidence was used to support a testing motion that led to the examination and testing of multiple items from the crime scene, the development of 24-plex Short Tandem Repeat (STR) and Y-Filer® profiles that confirmed the DQ Alpha and Polymarker testing and the development of additional evidence that was used in post-conviction.

This presentation will include discussion of the lessons learned from this experience. This presentation will discuss finding a qualified DNA lab that can apply outdated technologies to newly discovered evidence and how to develop new forensic evidence from old and degraded samples to advance your case, including the limits of the new technologies. This presentation will suggest strategies to strengthen post-conviction testing motions including the use of bloodstain pattern analysis to inform testing decisions and support the significance of DNA analysis.

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**Investigation, DNA, Defense**



### **F41 The Forensic Use of Closed-Circuit Television (CCTV) Surveillance Cameras in Italy: A Standard Procedure in Criminal Investigations to Enhance the Impact on Decision Making**

*Donatella Curtotti\**, Università di Foggia, Foggia, Puglia 71121, ITALY; *Sebastiano Battiato, PhD\**, Università di Catania, Catania 95125, ITALY; *Vittorio Rizzi\**, Direzione Anticrimine Polizia di Stato, Roma, ITALY; *Oliver Giudice, PhD\**, Roma 00044, ITALY; *Antonino Paratore, MS\**, ICTLab S.R.l. Spinoff of Università di Catania, Catania 95125, ITALY; *Wanda Nocerino\**, University of Foggia, Foggia, ITALY

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**Learning Overview:** The objective of this presentation is to share the Italian experience and its related protocol to improve the evidential use of images in criminal trials. In many states and jurisdictions, there are still no such rules and protocols.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through the increased competence in the use of images by investigators, as well as representing a good practice of collaboration between academics and investigators.

Often CCTV is a powerful tool in criminal investigations for many types of crime. Video and images contain important information on “who, when, and how” the crime has been committed. They also provide useful evidence in court. Today, the forensic value of video and images is limited by several factors. Those factors include the lack of a standard procedure for the collection by investigators and experts in a forensic way such that the evidence can be admitted at trial.

In Italy, there is no operative procedure on the use of images in criminal proceedings. Investigators do not have rules on identification, preservation, or the collection and analysis of video and images of CCTV surveillance cameras. Recent judgments of the Supreme Court have declared this type of evidence not admissible because it has not been handled in a forensic manner.

Because of the increasing use of images in criminal trials, Italian universities in collaboration with Polizia di Stato have drafted an investigative check list. Polizia di Stato (Italian State Police) asked the University of Foggia and the University of Catania to create an operating protocol to be distributed among its investigators throughout the country, after having subjected it to multiple verification processes to ensure its reliability.

This verification process has been thus standardized: (1) questionnaires distributed to all investigators of Polizia di Stato on their investigative habits on CCTV surveillance cameras; (2) elaboration of the questionnaires and drafting of the protocol by academic experts of criminal procedure and of computer science; (3) sharing of the protocol with prosecutor offices; (4) disclosure of the operative protocol through lectures presented to all investigators of Polizia di Stato; (5) experimentation of the operative protocol; (6) results of the experimentation and possible modifications of the protocol; and (7) official acquisition of the investigative protocol as an intervention guideline.

The protocol covers all stages of the investigation, including verification, identification and system description, preservation, collection and evidence acquisition, chain of custody, presentation, and reporting results. The parties involved are the investigating officer, the legal advisor, and a digital forensic expert.

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#### **Surveillance Cameras, Criminal Trials, Operative Protocols**



### F42 Dead or Alive: A Bioethics Approach to the Understanding of Brain Death Diagnosis by Families and Courts—A Report on Two Cases From the United States and Argentina

*Maria Susana Ciruzzi, PhD\*, Hospital Nacional de Pediatría Prof. Dr. Garrahan, Buenos Aires 1045, ARGENTINA*

**Learning Overview:** After attending this presentation, attendees will understand the challenges that come with a brain death diagnosis, and the dilemmas that surround decision making at the end of life with families, especially in cases of a child or a young adult. Attendees will see how the ethics of care shows a unique understanding of suffering and denial in families of a brain-dead patient and how poor and insufficient a simple legal approach can be, as it seems not to afford an answer that would overcome the despair and sadness of the patient's family. Attendees will also see the courts' understanding of a medical diagnosis and the difficulties legal professionals face when trying to analyze a medical decision from a legal approach.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing key aspects of the important role of bioethics in delivering bad news for families in medical situations at the end of life and the challenges professionals of the law, the courts, and the forensic sciences must deal with in order to guarantee surrogate decision making, the patient's dignity, and a good understanding of the medical diagnoses from courts.

Brain death is a hard diagnosis to make and even harder for families to accept. The idea of a corpse beating and breathing is extremely challenging to personal and religious beliefs. Fear always surrounds brain death diagnosis—the health team fears to err on the diagnosis and families fear to let go of their beloved one before his/her time.

The brain death definition states that death is “the irreversible cessation of all functions of the entire brain, including the brain stem.” This definition is found in the Uniform Declaration of Death Act (1981) of the United States and in the Organ Transplantation Act (24193) and the National Protocol for the Certification of Brain Death under Neurological Criteria, both from Argentina.

The diagnosis of brain death has important ethical, legal, and clinical implications. Although the concept of brain death is long-established criteria, how brain death is determined in practice can vary. These differences in practices can contribute to confusion, particularly on the lay public, but also in the courts.

Usually, a family's refusal to accept the diagnosis of brain death, the proper consideration of a patient's or a family's religious and personal views of death and perspectives on organ donation, and how accommodating hospitals should behave toward bereaved families can spark much controversy and debate. The goal is to present the challenges that a brain death diagnosis brings to health teams, families, and courts at the crossroads of medicine, individual rights, and a legal approach.

Brain death will be examined through two cases: Jahi McMath, a 17-year-old girl whose parents defied a brain death diagnosis, and the Ahumada Nuñez Case and the Argentine court's decision in a criminal case with a young brain death victim.

This presentation will show how juridification of clinical practice can lead to therapeutic obstinacy and can also result in a self-fulfilling prophecy: asking a judge about a medical decision that should have been decided between doctors and the patient/family, according to a legal and bioethical framework that opens the chance of legal scrutiny over doctors' decisions. Involving courts in clinical practice can lead to judicial obstinacy, can harm the patient's dignity, and shatter the family's deepest loving feelings.

Bioethicists in the clinical environment are tasked with being respectful to all parties when there is conflict, trying to reach a consensus in which agreement is quite challenging. Palliative care can be a better approach to help families cope with a brain death diagnosis and to help health care professionals do what is in the best interests of the patient.

This presentation seeks to provide a unique insight into how doctors and courts respond to the changing medicolegal culture and its consequences on patient care. The complexity of decision making at the end of life suggests a multidisciplinary approach, which must include training of health professionals and courts, as well as societal education and engagement.

This presentation will highlight the importance of ethical discussion by healthcare professionals about their everyday decision-making practice and how this can be influenced by changes in the law. This presentation will also raise questions about the ethical role of the law in the decision-making process.

This presentation will conclude that all that is needed is **diligence** in acquiring professional and scientific knowledge; **dedication** to the patient and family's needs, doubts, and fears; and **devotion** to truth, care, and empathy.

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#### Brain Death, Juridification of Clinical Decision, Bioethics



### F43 Justice Cannot Happen in a Vacuum: Toward a Better Representation of the Science, the Client, and the Case Through Open Communication

*Charlotte J. Word, PhD\**, North Chesterfield, VA 23235-0153; *Terri Rosenblatt, JD\**, The Legal Aid Society, Bronx, NY 10451; *Raymond Valerio, JD\**, Bronx County District Attorney, Bronx, NY 10451; *Heather H. Degnan, PhD\**, NMS Labs, Willow Grove, PA 19090

**Learning Overview:** The goals of this presentation are to educate analysts, prosecutors, and defense lawyers about each other's respective role and perspective on a criminal case to promote accurate and clear communications regarding scientific conclusions between the prosecutor, defendant, law enforcement, and the trier-of-fact (i.e., judge, jury). This presentation will demonstrate how open communication benefits each participant in a criminal case and ultimately best serves justice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of the roles and perspectives of the other participants in a criminal case and by providing tools to better communicate with each other. This presentation will impact attendees by increasing their competence and performance in their respective roles of representing the defendant, the government, or the scientific data through meaningful, open, and transparent communications.

**Hypothesis:** More effective, transparent, and well-informed communication between analysts, prosecutors, and defense lawyers about scientific findings aid in making critical decisions and lead to a better representation of the scientific data in criminal litigation.

The American system of justice is, by design, an “adversarial” system. Yet we are in an interrelated polygon of communication where we rely on information from each other to best do our respective jobs. The prosecutor must prove the government’s case beyond a reasonable doubt, and the defense attorney must present reasonable doubt to the finder of fact. As Chief Justice John Roberts wrote, “Federal prosecutors, when they rise in court, represent the people of the United States. But so do defense lawyers—one at a time.”<sup>1</sup> However, forensic scientists represent neither the “people” collectively, nor the defendant individually. Instead, they represent the scientific data in the case and the integrity of their laboratory’s work.

While the defense attorney’s and the prosecutor’s roles may be necessarily at odds, the forensic scientist is neither side’s ally nor adversary. Yet many analysts express concern about disappointing prosecutors or facing antagonistic cross-examinations by defense attorneys. These concerns, both by individual analysts and laboratory management, along with excessive workloads for all parties, can lead to policies that restrict communications between the scientists and the lawyers. Reports and testimony may communicate conclusions that comport with laboratory policies and procedures. However, restricted follow-up communications may lead to inadequate, or even misleading, representation of the data and their conclusions when the lawyers and/or fact finders are ill informed. This may ultimately result in unintended serious legal consequences (e.g., wrongful convictions, releasing the true perpetrator).

This presentation will explain the roles of the prosecutor, defense lawyer, and analyst in a panel discussion with members of each respective group. The defense perspective will explain the “one at a time” approach to defending the people of the United States and the constitutional duty to provide zealous advocacy for each client in each case. The defense panelist will discuss best practices for evaluating forensic evidence in constructing a theory of defense and advising an individual client. These goals are not aimed at discrediting sound science or making the analyst look foolish. Instead, they are geared toward contextualizing the science in a way that comports with the lawyer’s duties.

The prosecution perspective will explain the government’s need to understand the forensic evidence clearly. Once the prosecutor comprehends the forensic evidence, it can be presented unambiguously at all stages of the criminal justice process. How forensic evidence can inform charging decisions, plea negotiations, and trial strategy will be discussed. The importance of open communications with the analyst and the laboratory about the meaning of the conclusions and the manner in which the test was performed will also be discussed.

Ways in which laboratories can successfully work in an unbiased and neutral manner with everyone will be discussed. Best practices for reporting findings, providing effective testimony, and facilitating open communications will be presented. Examples of incomplete, misleading, or confusing report wording and testimony from actual case reports and transcripts will be presented and discussed.

This presentation will recommend steps that everyone involved in a criminal case can take to improve communications. Defense lawyers, consistent with their obligation to provide “effective assistance of counsel” must seek out training in forensic science and educate themselves before taking on a case. Prosecutors’ offices also must provide training to line attorneys in the capabilities and limitations of forensic science and how to accurately and fairly explain scientific evidence to a jury. Laboratories must openly and transparently provide information and encourage pre-trial meetings with all attorneys involved to ensure the data and conclusions, and their limitations, are effectively communicated.

#### Reference(s):

<sup>1</sup>. *Kaley v. United States*, 571 U.S. --- (2014).

#### Communication, Collaboration, Reports/Testimony



### F44 You Are the Judges! An Interactive Session in Forensic Medicine and Pathology on the Admissibility of Scientific Evidence in Shaken Baby Syndrome (SBS) and Abusive Head Trauma (AHT) Cases

W. Milton Nuzum III, JD\*, Supreme Court of Ohio, Columbus, OH 43215; Stephanie Domitrovich, JD, PhD\*, Sixth Judicial District of PA, Erie, PA 16501

**Learning Overview:** After attending this presentation, attendees will better understand the trial court's admissibility standards regarding expert testimony through interactive audience participation of the application of these standards in several case studies regarding forensic pathology issues.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing the knowledge of the factors for admitting scientific evidence in several specific case situations in the area of forensic pathology in both *Daubert* and *Frye* jurisdictions.<sup>1,2</sup>

Understanding the role of judges as gatekeepers will assist attendees in this session with comprehending and appreciating the critical admissibility decisions judges make as gatekeepers of scientific evidence in our courtrooms. *Daubert v. Merrell Dow Pharmaceuticals, Inc.* is the leading case regarding this gatekeeper role of admissibility decision-making.<sup>1</sup> The United States Supreme Court in *Daubert* defined the judge's role as a gatekeeper for admitting scientific knowledge to assist the triers of fact in understanding the evidence. Judges should admit conclusions that will qualify as scientific knowledge if the proponents of such evidence can demonstrate such conclusions are the products of sound scientific methodology derived from the scientific method. In order to be admissible, this evidence must be reliable and relevant. Understanding the expert's methodology is critical to effective, efficient, and proper judicial gatekeeping.

In explaining this evidentiary standard, the *Daubert* Court provided several factors for trial judges to consider: (1) whether a theory or technique can be (and has been) tested, (2) whether the theory or technique has been subjected to peer review and publication, (3) the known or potential rate of error of a particular scientific technique, (4) the existence and maintenance of standards controlling the technique's operation, and (5) a scientific technique's degree of acceptability within relevant scientific communities. The *Daubert* Court also emphasized that these five factors under Federal Rule of Evidence 702 are applied by trial judges in a flexible manner. Trial judges have great discretion in deciding on the admissibility of evidence under Federal Rule of Evidence Rule 702 and appeals from *Daubert* rulings are subject to a very narrow abuse-of-discretion standard of review.

Moreover, Federal Rule of Evidence 702 requires the following for expert testimony: A witness who is qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise if: (1) the expert's scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or to determine a fact in issue; (2) the testimony is based on sufficient facts or data; (3) the testimony is the product of reliable principles and methods; and (4) the expert has reliably applied the principles and methods to the facts of the case.

Also, under Federal Rule of Evidence 403, the trial judge may exclude relevant evidence if its probative value is substantially outweighed by the danger of unfair prejudice, confusing the issues, misleading the jury, undue delay, wasting time, or needlessly presenting cumulative evidence. Federal case law recognizes certain situations call for exclusion of evidence that is of unquestioned relevance. These situations entail risks ranging from inducing decisions on purely emotional bases to confusing or misleading jurors. Trial judges must balance the probative value of and need for this evidence against the harm likely to result from admitting said evidence.

Several state courts still apply the *Frye* standard instead of the *Daubert* standards. Trial judges in *Frye* jurisdictions in essence defer to the scientists and admit expert opinion based on scientific techniques only when the techniques are generally accepted as reliable in the relevant scientific community.

This presentation intends to focus this session on teaching participants to apply the trial courts' admissibility standards regarding expert testimony through the vehicle of interactive audience participation. This presentation will examine SBS and AHT cases under various court rules and case law to examine how courts make decisions on expert testimony in actual court cases. This presentation will address issues such as: How are SBS and AHT presented in the courtroom? Should expert testimony regarding SBS be admitted as scientific evidence to show that the baby's injuries were intentional? What are the clinical findings by experts in these cases? Is SBS considered by the courts as junk science? Can SBS cause fatalities and what does the scientific research indicate? Are these concepts acceptable in *Frye* states?

#### Reference(s):

<sup>1</sup> *Daubert v. Merrell Dow Pharmaceuticals*. US Supreme Court 509.U.S.579,113S.Ct.2786, 125L. Ed.2d 469. 1993.

<sup>2</sup> *Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923).

#### *Daubert*, Admissibility, Pathology



### F45 Forensic Validation, Error, and Reporting: A Unified Approach

Mark W. Perlin, PhD, MD\*, *Cybergenetics, Pittsburgh, PA 15213*

**Learning Overview:** After attending this presentation, attendees will understand how validation studies and match error are two sides of the same coin. Trial lawyers can apply these methods to ensure DNA evidence reliability in the courtroom.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping trial lawyers better understand and explain DNA limitations in criminal justice.

Federal Rule of Evidence (FRE) 702 requires reliable scientific evidence from expert witnesses. The *Daubert* standard specifically includes prongs for validation testing and error rate determination.<sup>1</sup>

Simple DNA evidence comes equipped with a built-in false positive error rate, the Random Match Probability (RMP) that states the chance of adventitious match to an uninvolved person. More complex evidence arises with small amounts of DNA or mixtures of several people. To address uncertainty, Likelihood Ratios (LRs) are used to quantify match strength.

Validation studies measure DNA match information on representative data sets. Match specificity is assessed by comparing many uncertain genotypes with many unrelated references, producing a large set of non-matching LR values. A histogram of these numbers on a logarithmic scale shows the log(LR) distribution of non-matching comparisons. These many-to-many, evidence-to-reference comparisons provide a frequency framework for assessing a laboratory's mixture data or interpretation method.

The match specificity histogram can be used to estimate the false positive error rate in an individual case. Comparing an evidence genotype with a suspect reference yields a match statistic. Positioning this log(LR) match value along the histogram shows how often a false match would occur. The validation fraction exceeding the reported log(LR) value is an error estimate of the Probability of Misleading Evidence (PME). PME generalizes RMP to handle inexact DNA matches.

The specificity histogram can be *directly* constructed from a validation's probabilistic genotypes, *without* reference sample comparisons. Information theory lets a computer assemble a composite log(LR) distribution from the distributions of many uncertain genotypes. This elegant, many-to-none approach accelerates validation studies.

A log(LR) specificity histogram can also be developed for a single evidence item. Older statistical approximations construct a one-to-many comparison of the uncertain genotype against many unrelated references. However, a more precise one-to-none *direct* construction immediately computes a numerically *exact* genotype specificity distribution solely from the evidence genotype, without making any reference comparisons. This direct approach is fast and accurate and enables routine error reporting.

The log(LR) specificity distribution is inherent in a genotype's probability description. Comparing evidence with a suspect produces a log(LR) value. Positioning this reported match value within the frequency context of the specificity distribution yields an error rate for false match. The fraction of specificity matches exceeding the suspect's statistic provides the PME—the chance this evidence would be as strong against someone who didn't leave their DNA. This error rate is based on the actual DNA evidence in the case, not on a validation study done at another time and place by other people on unrelated DNA samples under idealized laboratory conditions.

For a reported match statistic, FRE 403 would suggest that a focused, evidence-derived error rate is more relevant than a generic, validation-derived error rate. Reporting on facts in evidence has greater probative value. A more germane, evidence-based error rate can better assist a trier of fact.

Probability is readily communicated with whole numbers (for example, as “one in N people would match the evidence as strongly as does the suspect.”)

With smaller match statistics (e.g., under a million), reporting PME error provides a frequency context for understanding what LR information means. Moreover, a numeric error is more precise than a verbal equivalent.

An exclusionary match statistic also has PME error. Defenders can use these false negative error rates to contextualize exculpatory DNA evidence.

This presentation provides a unified view of evidence and validation error rates. Case examples show how inclusionary and exclusionary match statistics, along with error rates, are presented in court. Direct error determination for evidence items may reduce the need for large-scale validation studies. Normative science reports relevant error for measured variables. *Daubert* encourages such error reporting in forensic science.

A unified approach to forensic validation and match error will be presented. This presentation will offer useful perspectives on DNA evidence reliability.

#### Reference(s):

<sup>1</sup>. *Daubert v. Merrell Dow Pharmaceuticals*. US Supreme Court 509.U.S.579,113S.Ct.2786, 125L. Ed.2d 469. 1993.

#### Mixture Validation, Match Error, Forensic Reporting





**F46 WITHDRAWN**



### F47 Caught by the Laws of Physics

*Peter J. Diaczuk\*, Penn State University, State College, PA 16802; Xiao Shan Law, BS, Pennsylvania State University, Brooklyn, NY 11223*

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**Learning Overview:** After attending this presentation, attendees will understand a specific event, including several anomalies that led to controversial theories about the event, and how high-speed photography elucidated a serious misunderstanding of firearm dynamics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how an objective assessment of the evidence supported one of the competing theories of the case.

The death of a young woman several years ago in St Augustine, FL, was investigated by local law enforcement agencies. During the investigation, there was controversy regarding whether her death would be pronounced a suicide or homicide. She was killed by a single shot into her mouth by a .45 caliber handgun. The young woman also sustained an injury directly above her eye, which was just one of many anomalies in this case that initiated considerable controversy between those who supported the suicide theory and those who supported the homicide theory. One preposterous theory about the cause of the victim's eye injury was that it was caused by the firearm lurching forward, in the same direction of the bullet, upon being fired in what may be described as "negative recoil." The firearm had a tactical flashlight mounted on its proprietary under-rail, with a knurled bezel surrounding the parabolic reflector. The knurled bezel was very likely the cause of the victim's eye injury, but the question of how and when it happened needed to be explored. The Laws of Physics clearly prevent the firearm from moving forward upon being discharged, but sometimes the Laws of Physics are not well understood by those unfamiliar with them. Fortunately, high-speed photography can clarify very fast events that may otherwise evade understanding, or skepticism, in the tenets of science.

One theory of the incident proposed that the firearm lurched forward upon firing, which resulted in the bezel of the attached tactical flashlight to cause an arc-shaped injury over the victim's eye. That scenario would require the firearm to be used upside down to fire the fatal shot into the victim's mouth, almost simultaneously causing the eye injury with the aforementioned "negative recoil." This clear breach of physics can easily be visualized and disproven using high-speed photography, which was used in this project to elucidate the relative motion of the firearm and the bullet. The same model firearm and ammunition were used during several experiments concerning recoil, including supporting the firearm on strings and firing it remotely. A review of the frame-by-frame images taken during tests when the firearm was discharged revealed the bullet left and completely cleared the barrel before the slide began to move rearward. It only moved *rearward*, opposite the direction of travel of the bullet, not forward. This rearward-only motion of the firearm upon discharge obviously included the attached tactical light, contradicting notions that the tactical light caused the victim's eye injury at the same time that the fatal bullet was fired.

Other anomalous aspects of this event that will be discussed include two shots being fired at the scene and the locations of the two discharged cartridge cases with respect to the position of the firearm. Attendees may draw their own conclusions about this event after the facts are presented.

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### Suicide, Homicide, Recoil



### F48 Recent Legal Measures Against Stalking in India

*Anil Aggrawal, MD\*, Maulana Azad Medical College, New Delhi 110002, INDIA*

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**Learning Overview:** After attending this presentation, attendees will have learned the latest legal measures against stalking in India. Stalking is a widely prevalent problem and an attempt will be made to present a comparative aspect of laws in different parts of the world, giving attendees an insight into the strengths and weaknesses of legal measures against stalking in different jurisdictions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by stimulating further inquiry and research into the specific sexual behavior of stalking. Attendees will be educated about the problem of stalking in India and the recent legal measures that have been taken against this practice. The impact of this new law on the practice of stalking will be discussed.

**Background:** Stalking refers to a constellation of repeated and persistent behaviors to impose unwanted communication or contact on another person. Communication can be via emails, graffiti writings, letters, paper notes, telephone calls, or texts. Unwanted contact can be appearing in places the victim is expected, approaching the victim, following the victim, maintaining surveillance, and visiting the victim's home, family, or friends. Additional behaviors may include ordering goods or canceling appointments on the victim's behalf or initiating bogus legal actions. Physical assaults, property damage, and threats may accompany stalking. Approximately 80% of stalking is done by men.

**Content:** The Government of India has now introduced a new section, s354 D, in the India Penal Code, which is far more strict than that prevalent in many other parts of the world. On the first conviction, there is imprisonment (simple or rigorous) of up to three years and a fine (any amount.) On the second or subsequent conviction, there is imprisonment (simple or rigorous) of up to five years and a fine (any amount). The law against stalking in India and some recent case studies will be discussed. This law will be compared and contrasted with those in other parts of the world. Sometimes stalking is followed by rape, sexual assault, and/or murder. If these crimes are committed, more sections of law are attracted, and the entire crime becomes more complex. In India, it is seen that victim responses, although quite normal, may inadvertently elevate risk. A victim may respond aggressively or disrespectfully to an obsessional follower out of frustration or fear, unintentionally wounding the subject's fragile ego. Victims may fail to take the safety suggestions of law enforcement and demonstrate a lack of security consciousness. Forensic counselors are now advising victims of stalking not to respond aggressively or disrespectfully. This is one of the measures suggested to attendees, so they can provide this type of counseling in their own countries.

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### Abnormal Sexuality, Stalking, Sex Crimes



### F49 Law N. 24 of March 2017 and the Italian National System of Guidelines (SNLG): Bureaucratization of Medicine in the Name of Security of Care or a Spending Review?

*Federico Patanè, MD\*, Catania 95123, ITALY; Massimiliano Esposito, MD, University of Catania, Catania 95123, ITALY; Martina Fichera, MD, University of Catania, Catania 95123, ITALY; Pasquale Malandrino, MD, University of Catania, Catania 95123, ITALY; Orazio Cascio, MD, University of Catania, Catania, ITALY; Monica Salerno, MD, PhD\*, Department of Forensic Pathology, Foggia 71121, ITALY; Cristoforo Pomara, MD, PhD\*, Catania, ITALY*

**Learning Overview:** The purpose of this study is to review the clinical and judicial implications after the approval of Italian law N. 24 from March 24, 2017.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by drawing attention to the fact that any attempt to bureaucratize medicine could lead to reduced trials, but also to worse treatments, slowing down scientific research, and relegating the medical personnel as a mere executor of guidelines without taking into consideration the specificity of each patient.

In recent decades, the medical error and the risk management action required to prevent organizational dysfunctions and litigation regarding medical malpractice (and anything else that could compromise the security of care) has increased exponentially, becoming a controversial phenomenon.

Although the first to introduce this was the English jurist Sir William Blackstone in 1768, who in his Commentaries on the Laws of England (1768) coined the term *mala praxis* (which then became malpractice) referring to medical activity, it is only from the 1980s (and almost exclusively in the United States) that the medical error and its consequences became a “problem: for medicine.

The Italian national health care system has suffered from many claims and judiciary trials against health care professionals, thus implying significant economic losses and wasting of resources.

The law N. 24 from March 24, 2017, was an attempt to address these issues, trying to limit these trials by the optimization and improvement of the quality of health care services. Furthermore, it tried to fasten the official recognition of guidelines and scientific literature and optimize the trials if the health care professional adopts these guidelines. Without the mentioned recommendations, the health professionals should adopt the best clinical practices.

The guidelines are recommendations defined by scientific evidence, with the goal of ensuring the appropriateness of a clinical choice. This is the reason it is not considered a dogmatic truth, that is an imperative one, but a conventional truth decided by a community of experts.

The new law attempts to reach these goals through the establishment of a National System of Guidelines. Any new guideline needs to be drafted by a recognized group, institution, or organization before being submitted and published on a nationwide scale. Every guideline then needs to be updated every two years. In addition, the law tries to minimize lawsuits by requiring the injured patients to attempt to reach an agreement with the medical personnel before appealing to courts.

These strategies lead to many questions and debates: Is a national overview the right approach in keeping the guidelines up to date? What if a new, better, international, and well-recognized guideline is released, but the national guideline is still obsolete and disagrees, even if only partially, with the international guidelines? Which guideline should the health care professionals follow? Why should a health care professional take the responsibility to choose the best treatment for the patient, when the guidelines approved by the Ministry of Health can relieve him from responsibility, if this could cause further damage to the patient? Do these approaches really reduce litigation?

This law, while protecting the doctor from his responsibilities, seems to institutionalize the negative phenomenon of defensive medicine in a mass effect and to create the conditions for a national “cook book medicine,” which should be considered in view of two dangerous connected scenarios. The patient could no longer be treated as such, but only because of the professional risk it represents, and the physicians/researchers could have permanent loss of any interest in research.

The debate about law N. 24 from March 24, 2017, is still open and far from reaching its conclusion.

#### Professional Liability, Guidelines, Medical Malpractice



## **G1 Child Abuse and Neglect: A Forensic Odontological Approach**

*Salem Altalie, Abu Dhabi Police GHQ, Abu Dhabi, UNITED ARAB EMIRATES; Patrick W. Thevissen, PhD\*, KULeuven, Leuven, Vlaams-Brabant B-3000, BELGIUM*

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**THIS ABSTRACT WAS NOT PRESENTED.**



## G2 Forensic Odontology Education in Indonesia

*Nurtami Soedarsono, PhD\**, Faculty of Dentistry Universitas Indonesia, Jakarta, DKI Jakarta 10430, INDONESIA; *Masniari Novita, PhD*, Faculty of Dentistry Jember University, Jember 68121, INDONESIA; *Elza I. Auerkari, PhD*, Faculty of Dentistry Universitas Indonesia, Jakarta 10430, INDONESIA

**Learning Overview:** The goal of this presentation is to describe the development of forensic odontology postgraduate education in Indonesia. After attending this presentation, attendees will understand the importance of further education in the field of forensic odontology closely linked to the enforcement of human rights and law enforcement in Indonesia.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the need for the development of appropriate training modules to provide forensic odontology practitioners who are credible, experienced in the field of forensic odontology, skilled in handling cases, and able to provide expertise in a court of law. By understanding a comprehensive forensic odontology training module, it is expected that forensic odontology experts will have the ability to implement such training in the development of forensic odontology education in their respective countries.

Indonesia is an archipelago country located in the Ring of Fire at a meeting of several tectonic plates. This position makes Indonesia prone to both natural and man-made disasters. Under these conditions, Indonesia needs a large number of forensic experts, including forensic odontology experts. The role of a dentist becomes important as a first-responder resource as well as forensic expert.

The first postgraduate forensic odontology program in Indonesia was established in 2012. This master's program is designed to: (1) provide students with a general view of forensic pathology and a broad knowledge of forensic odontology; (2) develop students' analytical ability by conducting research in forensic odontology; (3) create professionalism in the field of forensic odontology; and (4) prepare students to pursue careers related to forensic science and have a good networking forensic organization. Lectures, laboratory exercises, and research are delivered to complete this program. In 2015, the Indonesian Dental Association established a forensic odontology college, mandated to formulate a specialist curriculum in the field of forensic odontology. This specialist program differs from a master's program, with an emphasis on students' skill development in forensic odontology. This program aims to: (1) provide students with a broad knowledge of forensic odontology; (2) train and develop students' skill in technical procedures of forensic odontology; (3) involve students and develop their skills in collaborative forensic investigations; (4) create professionalism in the field of forensic odontology; and (5) encourage students' involvement in national/international forensic organization networking. This specialized course is implemented through lectures, laboratory and clinical trainings, and casework. Collaboration with experts in the field of forensic pathology is indispensable in the forensic odontology postgraduate programs. Additionally, a collaboration with the police, disaster management agencies, and other relevant parties can support this quality education and provide students the best training.

### Forensic Odontology, Postgraduate Program, Training Modules



### G3 A Review of the Published Research on the Use of Overlays for the Analysis of Bitemarks in Skin

John P. Demas, DDS\*, Brooklyn, NY 11209

**Learning Overview:** After attending this presentation, attendees will understand the published research to date relating to the ability of the forensic odontologist to accurately interpret bitemarks on skin in regard to their source and the scientific support for said interpretation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by advancing the discussion of the use of bitemark analysis in the courts.

The hypothesis that human or animal dentitions can leave identifiable patterned impressions on skin (human or otherwise) after having bitten said substrate seems intuitive. The underlying assumption is that individual dentitions are just that—unique. For the purposes of this presentation, that assumption will be a given, as this presentation will not discuss the literature in support or opposed to the unique nature of dentitions but will simply stipulate that human dentitions are unique.

Carrying the hypothesis one step further, it had been put forth that one could identify the originator of a bitemark (the biter) via analysis of the shape and pattern of the mark and comparison with overlays of dentitions of the individuals who may have made the mark.

Research to investigate this hypothesis was undertaken in the 1970s, then after a nearly two-decade hiatus, research began again in the 2000s. In the intervening years, most of the published articles referencing bitemarks dealt with individual cases or an odontologist's preferred methodology rather than an exploration of the validity of the hypothesis or the faithfulness or reliability of skin (human or porcine) as a recording medium. This change of focus may have been due to the notoriety afforded odontologists after the Ted Bundy conviction and/or the enthusiasm with which bitemark analysis was embraced by law enforcement in this country.

This presentation will offer a summary of the research performed, the conclusions, and the recommendations of this study.

The 2009 publication of the National Academy of Sciences Report, *Strengthening Forensic Science in The United States, A Path Forward*, noted in the Summary Assessment of bitemark analysis that: "Although the majority of forensic odontologists are satisfied that bitemarks can demonstrate sufficient detail for positive identification, no scientific studies support this assessment, and no large population studies have been conducted. In numerous instances, experts diverge widely in their evaluations of the same bitemark evidence, which has led to questioning of the value and scientific objectivity of such evidence ... the committee received no evidence of an existing scientific basis for identifying an individual to the exclusion of all others. That same finding was reported in a 2001 review, which 'revealed a lack of valid evidence to support many of the assumptions made by forensic dentists during bitemark comparisons.' Some research is warranted to identify the circumstances within which the methods of forensic odontology can provide probative value."<sup>1</sup>

In addition to reviewing the above-captioned research, this presentation will touch upon the topic of wrongful convictions and why they may have occurred, and an alternative path forward will be offered.

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**Bitemark Research, Hollow Volume Overlays, Wrongful Conviction**



## G4 Bitemark Moratorium Part 1: Past Culture and Present Thinking

Cynthia Brzozowski, DMD\*, Sea Cliff, NY 11579; Robert E. Wood, DDS, PhD, Ontario Forensic Pathology Service, Toronto, ON M5G 2M9, CANADA; Franklin D. Wright, DMD\*, Hamilton County Coroner's Office, Cincinnati, OH 45230

**Learning Overview:** After attending this presentation, attendees will understand: (1) the failures of bitemark evidence in some of the wrongful conviction cases in the United States, (2) the recommendations made by the Texas Forensic Science Commission, (3) the current scientific research, and (4) a potential outline for empirical bitemark studies as a path forward.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that the culture and evolution of bitemark thinking has changed greatly since the publication of the National Academy of Sciences Report in 2009 and that the research to support more current bitemark guidelines remains absent.<sup>1</sup> This presentation will illustrate the fundamental issues of bitemarks that require scientific validity and, potentially, how this may be accomplished.

The “comparison sciences”—forensic feature comparison methods in criminal cases in the United States—have come under increased scrutiny and pressure from both the scientific and legal communities. In large part, this is due to the lack of a scientific underpinning, examiner reliability, and validation. Bitemarks are included in the category of subjective feature comparison methods that are based on examiner interpretation and experience. Three independent scientific panels—the National Academy of Sciences (NAS), the President’s Council of Advisors on Science and Technology (PCAST), and the Texas Forensic Science Commission (TFSC)—have all concluded that there are no scientific studies that validate examiner reliability or establish accuracy of bitemark analysis and comparison methods.<sup>1-3</sup> Proficiency testing is essential for all fields of forensic science, yet remain absent in bitemark analysis and comparison methodology. Calculation of an error rate is essential for assessing probative evidentiary forensic value in criminal cases in which an individual’s life, placement of minor children, and liberty is at stake.

In April 2016, after a six-month investigation by the TFSC, the panelist of scientists and attorneys recommended a moratorium on bitemarks in criminal cases in the state of Texas pending further research demonstrating sufficient examiner reliability and validity. The commission’s decision was a result of a comprehensive review of the past and current bitemark literature as well as testimony heard from several American Board of Forensic Odontology (ABFO) Diplomates and others within the scientific community. The shockingly poor results of one study constructed to examine the most basic aspect of bitemarks and to assess levels of agreement in determining if patterned injuries in skin were made by teeth or not captured the attention of the Commission. If there are not reliable criteria to define a pattern injury as a bitemark, then trying to analyze and compare the pattern to suspect dentitions cannot be scientifically supported.

The number of wrongful convictions and indictments based on bitemark evidence in the United States currently stands at 31. Some believe the ABFO has addressed the underlying failures of these cases by changing their standards and guidelines accordingly. This may be a good first step. However, in a recent 2018 editorial in the *Journal of Pathology and Medicine*, several authors wrote: “Understanding the causes and attempting to clarify where, how, and why the wrongful convictions occurred is necessary to be able to take measures to reduce the likelihood of such failures from happening again.”<sup>4</sup> This is a worthy goal that can only be partially fulfilled by looking backward at errant cases. The NAS Report and the independent scientific panels have said that there needs to be scientific data to support the recognition, analysis, and, where appropriate, comparison of bitemark patterns’ attribution to suspect dentitions.

This presentation will address some of the bitemark evidence and expert testimony in four recent wrongful convictions, review the current status of bitemark methodology, and outline a potential science-based path forward.

### Reference(s):

1. Committee on Identifying the Needs of the Forensic Science Community, National Research Council of the National Academies. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press, 2009.
2. The President’s Council of Advisors on Science and Technology (PCAST) Final Report. *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*. September 2016.
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### Bitemark, Moratorium, Wrongful Convictions





## G5 Bitemark Moratorium Part 2: Moving Forward

Cynthia Brzozowski, DMD, Sea Cliff, NY 11579; Robert E. Wood, DDS, PhD\*, Ontario Forensic Pathology Service, Toronto, ON M5G 2M9, CANADA; Franklin D. Wright, DMD, Hamilton County Coroner's Office, Cincinnati, OH 45230

**Learning Overview:** After attending this presentation, attendees will better understand the current status of bitemark methodology and a potential outline for empirical studies as a path forward.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the fundamental issues of bitemarks that require scientific validation via research. Until and unless proven methods in bitemark identification, analysis, and comparison can be scientifically established, a moratorium on the use of bitemark evidence in criminal cases should be in place.

Guidelines and standards established in the 1970s by the American Board of Forensic Odontology were based on the experience, knowledge, and training of the bitemark examiners. While the language and terminology has evolved over time, a scientific underpinning of the methods used in bitemark pattern identification, analysis, and comparison still do not exist.<sup>1-3</sup> When the Federal Rules of Evidence adopted the *Daubert* Standard, bitemark evidence grandfathered in by historically being recognized as generally accepted by past court rulings as precedent. In today's environment, "generally accepted" evidence based on "experience, knowledge, and training" is no longer acceptable without a quantitative empirical scientific underpinning. Past wrongful convictions and indictments in the United States, Canada, the United Kingdom, and other countries have shown that opinions of even the most experienced forensic dentists are fallible.

A bitemark literature search from 1966 to 2006 revealed that only 15% of all published papers were empirical studies. The vast majority were case reports of human bitemarks.<sup>4</sup> Previous empirical studies conducted to assess error rates in bitemark analysis using porcine skin have problems with scientific external validity.<sup>5,6</sup> Only a few studies have been conducted using "real-life" bitemarks to assess levels of expert training on interpretation of bitemarks and comparisons to a dentition.<sup>7-11</sup> Though the level of disagreement among forensic dental experts is troublesome, some studies show that expert disagreement is related to the quality of the evidence.<sup>12</sup> Each research study has its flaws, but to be able to move forward, the following basic questions need to be answered: (1) Can a pattern injury be reliably determined to be a bitemark or not?; (2) How does one determine whether a purported bitemark possesses sufficient evidentiary value to warrant further investigation or comparison?; and (3) Can a given dentition be excluded or not excluded as having made the bitemark?

This presentation will highlight a path forward for proposed studies whose goal is to provide a framework that may or may not establish justification for the recognition of bitemark patterns, their analysis and, where appropriate, comparison to suspect biters. Lacking this scientific underpinning, bitemark pattern injury attribution to a suspect should not be introduced as evidence in criminal cases going forward and even bitemark recognition should be undertaken with caution.

### Reference(s):

1. Committee on Identifying the Needs of the Forensic Science Community, National Research Council of the National Academies. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press, 2009.
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### Bitemark, Moratorium, Empirical Studies



## G6 What We Can Learn From Cadaver, Volunteer, or Actual Case Material in the Analysis of Bitemark Evidence Using the Full Spectrum of Photographic Techniques

Richard R. Souviron, DDS\*, Coral Gables, FL 33134

**Learning Overview:** After attending this presentation, attendees will learn the value of different modalities of experimentally produced bitemarks, the analysis of each type of experimental bitemark, and its value when applied to “real-life” cases. The full spectrum of forensic photographic techniques will be shown and their advantages for use in real-life bitemark analysis.<sup>2-4,5-7</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the full spectrum of forensic photographic techniques and their advantages for use in real-life bitemark analysis.

The limitations of laboratory bitemark research techniques vis-a-vis actual case material will provide some useful objective evidence for practical application. When applying the different forensic photographic techniques to the laboratory-produced bitemarks and evaluating the results, one will learn the value of each of these techniques.<sup>1,7,6</sup> Then, when applying these results to actual bitemark cases, there should be improvement in the final analytical results.

To date, laboratory research with bitemarks has been limited to producing bitemarks on cadavers, anesthetized fetal pigs, and live human volunteers. This has been done with plastic teeth mounted on a mechanical device (vice grips) using hand/arm pressure with a vertical closure on a non-moving subject. In all this research, there is no movement on the part of the subject, real teeth are not used, and the muscles of mastication are only approximated. These experiments, although providing some valuable information, are not a real-life duplication of bitemarks inflicted by the victim on the attacker or by the attacker on the victim.

The results of laboratory bitemark research can be augmented by using ultraviolet light, Alternate Light Source (ALS), in addition to ambient light for the enhancement of surface detail and Infrared (IR) light to help analyze wound depth.<sup>2-4,6</sup>

The basic information from laboratory-produced bitemarks on cadavers, fetal pigs, and human volunteers, along with the full-spectrum forensic photographic techniques, when applied to real-life bitemark cases, should produce increased accuracy and be based on some degree of scientific research.<sup>1,4,5</sup>

The analysis of real-life bitemarks in human flesh may produce valuable information for the authorities in the pursuit of justice. The comparison of the suspected biter’s teeth to the bitemark left on the victim should be tempered with the many variables that occur in the actual situation.<sup>1,2,5,7</sup> The distortion that occurs due to many various factors, such as the movement of both parties, the elasticity of the skin in the area bitten, and the amount of tissue bitten.

The one constant in bitemarks is that the DNA of the biter may be obtained. In many cases, this can make an identification or an exclusion to a reasonable degree of certainty. However, the analysis of the pattern injury can provide useful information not only for exclusion or inclusion of the biter, but other information, such as position of the biter, adult versus child, age of the bite in relation to time of death, etc. The addition of forensic photographic techniques can enhance such an analysis.<sup>3,5-7</sup>

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### Reflective Ultraviolet, Analysis, Comparison



## G7 A Bitemark Case

*John Berketa, PhD\*, University of Adelaide, Norwood, South Australia 5067, AUSTRALIA*

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**Learning Overview:** After attending this presentation, attendees will gain an appreciation that, in carefully selected cases, bitemark analysis may still play a role in bringing justice and allow some sort of closure to the relatives of victims.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by balancing recent media and board announcements regarding bitemark analysis that proved to be useful.

Cases involving bitemark analysis have lately been embroiled in controversy. The 2009 Report from the National Academy of Sciences and the President's Council of Advisors on Science and Technology (PCAST) have been scathing in the conclusions reached from poor bitemark evidence presented to courts of law. Following DNA evidence and work by the Innocence Project in the United States, people wrongly convicted have been rightly released. More recently, the Texas Forensic Science Commission suggested the halting of bitemark evidence until more scientific proof is provided.

A case involving the homicide of a young female victim will be presented, including the scenario at the scene, the initial investigations taken, the preliminary report, and the analysis of the images. Following queries from the police, further investigations undertaken that could possibly assist jurors in understanding the dynamics of the case will also be presented. The final report with the conclusions given will also be presented.

The outcome of this case led to the conviction of the suspect in which DNA evidence was restricted due to the accused proactively destroying evidence that could have produced samples and a delay in the discovery of the body. The accused received a life sentence with a minimum 25-year non-parole period. The absence of bitemark evidence in this case would have most likely allowed the dismissal of the charges against the accused.

The clear majority of possible bitemark evidence presented to odontologists is of poor quality for an analysis to be undertaken. The pressure exerted on forensic odontologists from legal authorities to analyze these possible injuries may be great and, together with emotional bias, may lead to a situation in which the conclusions are overstated. Poor evidence should not be accepted and stated as such in a preliminary report. However, if the evidence is suitable in quality to be investigated, and appropriate legal procedures followed, it would seem to be a miscarriage of justice not to present the evidence to the court.

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### **Bitemark, Analysis, Conviction**



### G8 From Gerontophilia to Fruit Biting: A Series of Bitemark Cases in Hungary

*Armin A. Farid, DMD\*, Budapest 1067, HUNGARY*

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**Learning Overview:** After attending this presentation, attendees will understand that bitemarks are found in certain rare cases and continue to provide reliable additional evidence in crimes involving murder, rape, abuse, and even burglary.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how biting occurs in every country. Public awareness, through the help of the mass media and continued education of first responders to recognize bitemarks, helps to bring to light these patterned injuries that assist as auxiliary evidence to solve a criminal act.

In addition to its long history in educational and theoretical fields in Hungary, human bitemark analysis is becoming ever more integrated into police investigation work today. Since 2008, continuous media campaigns and educational programs put into place for training Hungarian crime scene technicians have helped raise awareness of the importance of bitemark analysis among the police as well as the general public, resulting in the reporting of several bitemark cases within a short period of time. Nearly all private and public television channels have covered reports and documentaries on bitemarks, their analysis, and the implications in solving criminal cases, thus educating the public of their value in proving a suspect's guilt or innocence.

A recent murder case in the north of Hungary was of particular interest, as it involved the rape and murder of a 75-year-old woman by a 25-year-old man, who bit the victim's right breast, leaving a deep bitemark, which was only discovered during autopsy as the victim was presumed to have died of natural causes at home. While the investigation of this case was at full speed, in another town in central Hungary, a series of burglaries were investigated, resulting in the finding of a bitten apple at one crime scene and a bitten pear at another crime scene.

This study seeks to prove that the act of biting exists in every country. The importance of safeguarding accurate forensic evidence goes hand-in-hand with honesty and the dedication of experts who engage in the admirable work of public service in upholding the highest standards of ethics, truth, and justice.

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**Bitemark, Crime Scene, Gerontophilia**



### **G9 The Impact of the First International Forensic Odontology in Human Rights Workshop in the United Arab Emirates**

*Salem Altalie, Abu Dhabi Police GHQ, Abu Dhabi, UNITED ARAB EMIRATES; Emilio Nuzzolese, PhD\*, Dental Team DVI Italia, Bari 70124, ITALY; Joe Adserias-Garriga, DDS, PhD, Texas State University, Forensic Anthro Dept, San Marcos, TX; Alexander S. Forrest, MDS, Griffith University Nathan Campus, Nathan, Queensland 4111, AUSTRALIA*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the forensic odontologist's role in human rights, as well as the critical part of restoring human identity and dignity to unidentified human remains using dental information.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as an example of how to increase awareness of humanitarian forensic odontology.

A forensic odontology workshop was held at a forensic science conference in November 2017 at Abu Dhabi, United Arab Emirates (UAE). The conference focused on the latest innovations and challenges facing the forensic science community. The full-day workshop in forensic odontology was offered for the first time in the UAE by four international experts. It was organized by the Medical Examiner Office of Abu Dhabi Police in partnership with Forensic Odontology for Human Rights (FOD4HR). FOD4HR is an international, non-governmental group with more than 100 experts in forensic odontology that promotes best practice in human identification and human rights protection.

The topics covered for this study were age estimation, dental identification, anthropology comparison, and pattern injuries analysis and concluded with a panel discussion session. The workshop was designed to be theoretical through the examination and interpretation of reported cases. In addition, there were hands-on exercises to enhance skills and encourage the exchange of participants' experiences.

To assess the impact of the forensic odontology workshop, statistical analysis was based on a questionnaire completed by attendees to evaluate the effectiveness of the workshop. Only 12 participants were selected, out of 22, who work in the police force in the training department of Abu Dhabi Police. The demographics of attendees were nine general dentists, eight forensic scientists, three dental students, and two disaster victim managers.

As a result, there was a significant increase in the level of workshop learning objectives in knowledge, skills, and behavior by overall 41.14%, which increased from pre-workshop 32.81% to 73.95% post-workshop. The statistical results of the questionnaire revealed 94.44% participation satisfaction on the efficacies of the lecturers. In addition, 93.76% of the ability to execute the specific session on the topics were discussed.

In conclusion, the workshop revealed an overall of 93.75% impact on attendees. Furthermore, requests were made to broaden the scope of the topics discussed in forensic odontology, such as the role of odontology in migration and human trafficking. Forensic odontology can be a powerful resource for human rights anywhere in the world, which encourages the continual effort to conduct future humanitarian forensic odontology workshops.

#### **Human Identification, Human Rights, Humanitarian Forensic Odontology**



### **G10 Analysis and Identification of Bitemarks in Forensic Casework: Child Sexual Abuse**

*Carmen Silvia M. Miziara, MD, PhD\*, Sao Paulo 05409000, BRAZIL; Elaine S.N. Goto, Faculdade de Medicina do ABC, Santo André CEP: 09060-870, BRAZIL; Gabriela P. Ascani, Faculdade de Medicina do ABC, Santo André CEP: 09060-870, BRAZIL; Karen A.C. Fukuma, Faculdade de Medicina do ABC, Santo André CEP: 09060-870, BRAZIL; Thainá A. Marin, Faculdade de Medicina do ABC, Santo André CEP: 09060-870, BRAZIL; Daniela M. Abe\*, Faculdade De Medicina Do ABC, Santo André CEP: 09060-870, BRAZIL; Ivan D. Miziara, MD, PhD, Sao Paulo University School of Medicine, Sao Paulo 05406000, BRAZIL*

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**THIS ABSTRACT WAS NOT PRESENTED.**

## G11 Profiling an Unknown Castaway: “The Philly Cocoa Man”

John B. Nase, DDS\*, Dental Forensic Services of Indian Valley, Harleysville, PA 19438

**Learning Overview:** After attending this presentation, attendees will be able to outline how dental adult age assessment and georeferencing through chemical and elemental isotope analysis contribute to an unknown victim profile.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing how emerging anthropological isotope science can be used alongside time-tested dental age estimation techniques in some unknown decedent cases.

On May 11, 2015, the container ship *Sian-C* transporting cocoa beans from Côte d’Ivoire (Ivory Coast, Africa) docked in the Port of Philadelphia, PA. An unidentified black male was discovered in the cargo hold by United States customs during inspection and fumigation. Foodstuffs, water, and a set of clothing were adjacent to the body. No forms of identification were found within the cargo hold, and personal effects were limited to a jumpsuit and non-descript metal rings. Fingerprint processing was completed and run through both the Integrated Automated Fingerprint Identification System (IAFIS) and United States Homeland Security databases for undocumented workers, terrorist watch lists, and foreign nationals with no hits. The National Missing and Unidentified Persons System (NAMUS) submission has not produced a positive match to date.

The dental examination revealed no restorations or untreated gross decay. However, maxillary and mandibular acrylic, removable partial dentures were found. The results of three adult age estimation methods were in accordance with one another. Method 1, the Kvaal 1995 study, determined the decedent’s age as 26.57 years with a standard error of estimation of 8.6 years.<sup>1</sup> Method 2, the Cameriere 2007 study, determined the decedent’s age as 24.95 years with a standard error of estimation of 3.62 years.<sup>2</sup> Method 3, the Bang and Ramm 1970 study, determined the decedent’s age as 33.48 years with a 95% confidence interval of 9.46 years.<sup>3</sup>

A comparison of the isotope ratios of enamel verses bone samples can yield patterns of migration when an individual moves from one geographic region to another region through their lifetime.<sup>4-8</sup> This analysis was undertaken to confirm that the decedent was likely from the ship’s port of departure, to rule out the possibility that he gained access to the ship’s hold while in Philadelphia and further define his places of residence. Samples of teeth, mandibular bone, and rib were collected for georeferencing.<sup>9,10</sup> Both stable and heavy isotope analyses were completed for the following elements: Carbon ( $\delta^{13}C$ ), Oxygen ( $\delta^{18}O$ ), Strontium ( $^{87}Sr/^{86}Sr$ ), and Lead ( $^{206}Pb/^{204}Pb$ ,  $^{207}Pb/^{204}Pb$ ,  $^{208}Pb/^{204}Pb$ ).<sup>11,12</sup> The chemical and elemental isotope testing indicates the decedent was likely born outside of the United States. The enamel and bone data are consistent with an origin of birth in the African continent, as well as living in Africa the last several years of life.<sup>13</sup> When the strontium and lead isotope values are plotted on a bivariate graph, they fall closest to the known range of values from Nigerian enamel samples in comparison to data from the United States and Europe.<sup>14</sup> The  $\delta^{18}O$  values for the bone sample also fall within the West African region. Additionally, the  $\delta^{13}C$  values for decedent USF17-041C are consistent with the mixed C3 and C4 food crops found within West Africa. Overall, the findings are consistent with origin in West Africa, which includes Côte d’Ivoire and other countries in the region.<sup>15</sup>

A victim profile was compiled that consisted of reconstructive facial drawings, sketches of several tattoos found on the body, an anthropological examination, the dental age assessment, and isotope analysis results.

In conclusion, this case shows a multi-modal victim profile generated utilizing several technologies and forensic specialties.

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### **Dental Age Assessment, Isotope Analysis, Profiling**





### G12 When a 70-Year-Old Mummified Baby Falls on Your Head: Collaboration in a Multidisciplinary Team

*Corinne D'Anjou, DMD\*, LSJML, Saint-Lambert, PQ J4P1Y2, CANADA; Caroline Tanguay, MD\*, Montreal, PQ H2K 3S7, CANADA*

**Learning Overview:** After attending this presentation, attendees will understand the importance of a multidisciplinary team approach in an unusual medicolegal death investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how experts from multiple disciplines can work together, each bringing their own experience and expertise, in a team approach to efficiently dissect the evidence in a unique and challenging case.

The case began with the discovery of human remains during a remodel of the basement of a century-old house in Westmount, Quebec, Canada. The remains, wrapped in journal paper, appeared to be of a very young child or fetus. They consisted of an almost complete mummified fetus, still in a fetal position with its arms flexed and the lower limbs folded over the trunk and crossed.

Following an anthropological consultation, the lower limbs were moved, which exposed a portion of the umbilical cord. In the absence of internal organs and biological environment, the umbilical cord specimen, along with the right humerus and left femur, were submitted to the forensic biology section of the Laboratoire de Sciences Judiciaires et de Médecine Légale to be analyzed and potentially provide a biological profile to aid with the investigation. The fetus presented no evidence of traumatic injury.

Determining the gestational age of the remains and finding any other evidence that could lead to valuable clues as to the nature of the death and origin of the remains were critical to the investigators.

A forensic document specialist examined the several newspapers that were found with the remains to help determine a possible timeline surrounding the events. A daily schedule of a film that was originally released in 1948 was found within the newspapers. This discovery led the investigators to meet with a lady, now 78 years old, who lived in the house during that time. From interviewing her, they were able to get a better understanding of who inhabited the house around that time. Given the Hollywood-like history of the household and the multiple possibilities of parenthood, she agreed to provide a DNA sample to allow a comparison with the remains.

Forensic pathologists, anthropologists, and odontologists examined the remains visually and radiographically to provide an estimated gestational age utilizing various techniques, from the osseous centers in the hands and feet to the developing primary dentition.

In conclusion, with the multidisciplinary approach involving forensic pathologists, anthropologists, biologists, odontologists, and document specialists, the discovered remains were thoroughly examined, and an estimated age and DNA profile were provided to the investigators to help determine the origin, timeline, and circumstances surrounding the discovery of the mummified remains.

#### **Forensic Odontology, Age Estimation, Forensic Pathology**



### G13 Fetal Maceration and Dental Age

Mark W. Crumpton, DMD\*, Maryville, TN 37803; James M. Lewis, DMD, Madison, AL 35758; Darinka Mileusnic, PhD, MD, Regional Forensic Center, Knoxville, TN 37920; Murray K. Marks, PhD, University of Tennessee, Knoxville, TN 37920

**Learning Overview:** The goal of this presentation is to demonstrate fresh and decomposed fetal maceration techniques, tooth bud dissection from jaws, and age assessment from a dissected specimen using published standards.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by creating a methodology for recognizing, harvesting, and reporting on crown and root growth for age assessment in fetal viability.

This presentation provides traditional and expedited instructions for processing fetal remains, especially the oro-facial elements, to perform assessment of dental age. Because of their rare occurrence, the discovery of fresh, decomposing, disfigured, or skeletal fetal remains engenders a heightened awareness by the forensic pathologist primarily concerned with age in relation to viability. With decomposed, mutilated, or isolated remains, the anthropologist can assess “human” or “non-human” and will focus on long bone measurements and other skeletal maturity indicators to help assess age. Crucial for the pathologist: “Is this fetus beyond the age of viability and how long will it take to obtain an age assessment?” Equal to sub-adult remains, dental development is the most precise means of age assessment in the fetal skeleton. Dental age assessment involves: (1) dissection and maceration of soft tissues, (2) recognition of craniofacial bones and partially mineralized crowns in decomposing remains, and (3) excision and preservation of tooth buds for metric analysis.

Twelve fetuses ranging in age from 18 to 39 weeks were dissected and macerated using low power dissecting microscopy, magnifying loops, and lenses for limb, torso, and head removal. Parts were placed in glass jars with distilled water that was changed monthly. There was no heat or chemical treatment, which can compromise the structural integrity of the initial fragile mineralized tissues. Sloughed-off soft tissue was removed during decomposition by pouring off the water through a 14- and 18-gauge sieve. Cartilaginous epiphyses enlarge and separate easily from the metaphyses. Skull bones gradually separate along fontanelles. Extraction and wet preservation of tooth buds may be performed anytime during the soft tissue sloughing process. The dentoenamel junction separates easily and particular care is required to identify and retain the dentin. Teasing the crown out of the follicle is easier earlier in the decomposition process prior to later expansion of the follicle. Later, tooth buds may slough off with gingival tissue making identification more challenging. Dissolving fetal soft tissues is best achieved in the distilled water solution with complete skeletalization of a fresh fetus in four months. Sunlight exposure did not appreciably increase decomposition time. Decomposed “wet” remains are easier to dissect compared to desiccated remains that require extensive and careful dissection. Desiccated jaws and tooth buds need hydration to enlarge and loosen from hydrated alveolar bone. While any alcohol solution should be avoided because of tissue distortion, formalin submersion of the tooth buds will enhance preservation, though may render the follicle more tenacious to dissect. Kraus and Jordan age-assessed the largest sample of fetal deciduous teeth and that standard is still valuable today.<sup>1</sup> Partial to completely mineralized crowns, especially molars, are well-correlated to gestational age. Regardless of the condition of the jaws, a dissecting microscope, magnifying loops, lenses, and a good light source are used in combination to allow the dentist to perform the oral autopsy in a timely manner to provide the forensic pathologist an estimation of age.

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**Fetal Viability, Dental Age, Decomposition**

## G14 The Effects of Third Molar Impaction Parameters on Third Molar Development and Related Age Estimation

Rosalina Intan Saputri, DDS, MSc\*, Department of Imaging & Pathology, KU Leuven, Leuven, Vlaams-Brabant 3000, BELGIUM; Jannick De Tobel, MD, Ghent University, Department of Radiology, Gent, Oost-Vlaanderen 9000, BELGIUM; Patrick W. Thevissen, PhD, KU Leuven, Leuven, Vlaams-Brabant B-3000, BELGIUM

**Learning Overview:** After attending this presentation, attendees will understand how third molar impaction parameters affect third molar development and related age estimations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing proof that third molar impaction parameters have a clinically small effect on third molar development. This implies that in forensic practice, no distinction is necessary between impacted and non-impacted third molars for age estimation.

Third molar development is used to estimate age in subadults. However, the incidence of third molar impaction is high.<sup>1</sup> If third molars are impacted, their development may be affected, and it may have consequences for the performed age estimations.<sup>2</sup>

The goal of the current study was to evaluate the effect of third molar impaction parameters on third molar development and related age estimations. Parameters, such as contact between third and corresponding second molar, retro-molar space availability, and angulations between third and corresponding second molar, were examined on 5,279 panoramic radiographs. The radiographs were selected from 1,031 subjects (473 male, 558 female) with ages ranging between 3.2 and 23.5 years old. Based on the measured status of contact and angulations between third and corresponding second molar, an impaction status was assigned. All present third molars were staged according to the Köhler et al. staging technique.<sup>3</sup> A linear model was used to evaluate the difference in age between third molars with and without impaction. The model contained fixed effects of impaction (no/yes), stage, and sex. The same model was used to compare age as a function of contact between third and corresponding second molar and retro-molar space availability. For angulations between third and corresponding second molars, a quadratic function was used to describe the relation with age. The model has been fit separately for each third molar position. Significant differences were found in mean age as a function of presence of contact between third and corresponding second molar and retro-molar space availability, depending on third molar position and on stage. There was a significant linear relation between angulations and age, depending on stage. An impaction status was assigned to third molars in contact with the corresponding second molar and with angles between third and second molar  $< -22^\circ$  or  $> 18^\circ$  for the upper third molars and  $< -17^\circ$  or  $> 12^\circ$  for the lower third molars. There was a significant difference in age between impacted and non-impacted third molars. However, all the differences were clinically small ( $\leq 0.65$  years) and, for each third molar, the overall age of third molars with impaction was younger than third molars without impaction. Therefore, impacted third molars can be examined for age estimation purposes equally as non-impacted third molars.

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### Forensic Odontology, Dental Age Estimation, Third Molar Impaction

## G15 Age Estimation in Children and Subadults Combining Ratios of Pulp and Tooth Area With Tooth Development

Manasa Ravoori, BDS\*, KULeuven, Leuven B3000, BELGIUM; Jannick De Tobel, MD, Ghent University, Department of Radiology, Gent, Oost-Vlaanderen 9000, BELGIUM; Patrick W. Thevissen, PhD, KULeuven, Leuven, Vlaams-Brabant B-3000, BELGIUM

**Learning Overview:** After attending this presentation, attendees will understand why adding pulp and tooth ratios to tooth development on panoramic radiographs is of limited value for age estimation in children and subadults.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing why adding pulp and tooth ratios to tooth development on panoramic radiographs is of limited value for age estimation in children and subadults.

**Introduction:** Dental age estimation methods are mainly based on tooth development or on morphological changes observed in teeth.<sup>1</sup> In children and subadults, certain teeth are mature and start forming secondary dentin. Combining the age-related information of these secondary dentin formations and tooth development may improve the age prediction performances in children and subadults.

**Goal:** To detect if, in children and subadults, secondary dentin formation provides added age prediction information to tooth development.

**Materials and Methods:** Retrospectively panoramic radiographs of 453 subjects (228 females and 225 males) in the age range between 8 and 25 years were selected. The radiographs were imported in image-ameliorating software (Adobe® Photoshop®) to measure the pulp and tooth areas in the crown and the crown plus root portion of teeth 33 and 36 (Fédération Dentaire Internationale (FDI) nomenclature). For both teeth, the respective ratio between pulp and tooth area in the Crown (CR) and the Crown plus Root portion (CRR) were calculated. The development of the lower left seven permanent teeth and all present third molars were staged according to the Demirjian et al. and the Köhler et al. staging technique, respectively.<sup>2,3</sup> Within the group of children, it was verified for each of the ratios separately whether they contribute additional information to the age estimation based on the Willems et al. age estimation method.<sup>4</sup> To this purpose, a regression model for age was used with the prediction based on the Willems et al. method and the ratio as predictors. To evaluate the total dataset, the added value of the ratio information to the Kohler staging a similar approach was used. The age estimation based on the Kohler stages of the third molars was obtained applying Bayes rule with a multivariate continuation-ratio model as conditional distribution.<sup>5</sup> To quantify the age prediction performances of the models, the Mean Error (ME), Mean Absolute Error (MAE), and Root Mean Squared Error (RMSE) were calculated.

**Results:** Within the group of children, the CRR in tooth 36 significantly added information to the Willems et al. method, for males ( $p=0.0073$ ) as well as for females ( $p=0.0002$ ). However, the improvement in MAE was small and not significant (decrease in MAE=0.032 years (SD=0.303),  $p=0.09$ ). The CR for tooth 36 added significant information to the Willems et al. method within females, but the change in MAE was not significant. Neither for the CRR, nor the CR measured in tooth 33, was there any evidence that they contributed information to the Willems et al. method.

Neither for the CRR, nor the CR measured in tooth 36, was there evidence that they added information to the Kohler et al. stages. Only for males was the addition of the CRR significant, but this was not reflected in a significant decrease of the MAE. The MAE was significantly higher (but small) when adding the ratio information for females. Neither for the CRR, nor the CR measured in tooth 33, was there any evidence that they added information to the Kohler et al. stages.

**Conclusion:** On panoramic radiographs in children and subadults, pulp and tooth ratios were used as a measure of secondary dentin formation, but they did not provide added age prediction information to tooth development.

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### Forensic Odontology, Dental Age Estimation, Tooth-Pulp Area Ratios

## G16 The Influence of Lower Third Molar Segmentations on Automated Tooth Development Staging Using a Convolutional Neural Network Approach

Rizky Merdietio Boedi, MSc\*, KULeuven, Leuven B3000, BELGIUM; Nikolay Banar, MSc, KULeuven, Leuven B3000, BELGIUM; Jannick De Tobel, MD, Ghent University, Department of Radiology, Gent, Oost-Vlaanderen 9000, BELGIUM; Jeroen Bertels, Wilsele, Vlaams-Brabant 3012, BELGIUM; Dirk Vandermeulen, PhD, KULeuven, Leuven B3000, BELGIUM; Patrick W. Thevissen, PhD, KULeuven, Leuven, Vlaams-Brabant B-3000, BELGIUM

**Learning Overview:** After attending this presentation, attendees will understand how segmenting the lower third molar on panoramic radiographs affects automated stage allocation performance for age estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the consecutive steps needed in an automated age estimation procedure based on third molar development. In particular, the choice of an optimal tooth segmentation technique will be discussed.

**Introduction:** In an automated age estimation method based on third molar development, an automated developmental stage allocation technique can be integrated. De Tobel et al. established a pilot set up for such an automated technique, based on panoramic radiographs.<sup>1</sup> First, they manually selected a rectangular Region Of Interest (ROI) around the lower left third molar. Second, deep learning was used to further process the ROIs with a Convolutional Neural Network (CNN).<sup>2</sup> The network investigated a way to automatically allocate stages to the third molars. However, the used ROI contained information of surrounding anatomical structures (e.g., periodontal ligament, bony structures, inferior alveolar nerve canal). It can be assumed that segmenting only third molar information will reduce noise from these structures and will ameliorate the automated stage allocation performances.

**Goal:** To establish and validate an automated staging technique for third molar development after rough and full segmentation on panoramic radiographs.

**Materials and Methods:** Two human observers staged each lower left third molar in consensus, according to a modified Demirjian staging technique (ten stages).<sup>3</sup> A third observer acted as a referee in case of disagreement between the first observers. Per stage and per sex, 20 radiographs were included, and their stage classification acted as the gold standard. The images were imported in Adobe® Photoshop® CC 2018 and segmented using built-in tools. Three types of segmentation were processed: Box segmentation (B, similar to De Tobel et al., Rough tooth Segmentation (RS), and Full tooth Segmentation (FS).<sup>1</sup> CNN DenseNet201 from Keras API with TensorFlow backend was used for automated stage allocation in the three types of segmented images.<sup>4</sup> The automated staging was compared with the gold standard staging in general and per stage. Stage allocation performance was evaluated using a five-fold cross-validation between the automated and the gold standard stages, using 80% of the images for training and 20% for testing.

**Results:** The FS technique had the best overall results with 61% of correctly allocated stages, a mean absolute difference in stage allocation of 0.53 stages and a mean linear weighted kappa of 0.84. Per stage and for all segmentation types, the final root stages were the most difficult to distinguish from each other. The five-fold cross-tabulation indicated that misallocated stages were mostly neighboring stages.

**Conclusion:** Segmenting the third molar from the panoramic radiographs increased the overall stage allocation performance. The FS is advised to be integrated in an automated dental age estimation process.

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**Forensic Odontology, Automated Dental Age Estimation, Automated Tooth Development Staging**

## G17 Age and Sex Estimation of Ajnala Skeletal Remains From Trace Elemental and Radiographic Analysis of Molar Teeth: A Comparative Forensic Odontological Study

Jagmahender Singh Sehrawat, PhD\*, Panjab University, Department of Anthropology, Chandigarh 160014, INDIA

**Learning Overview:** The goal of this presentation is to estimate sex and age of the unknown human skeletal remains excavated from an abandoned well by using the customized regression and discriminant function formulas developed from elemental composition of molars and radiographic Pulp-Tooth area Ratio (PTR) of canines of known teeth. A comparative significance of elemental and radiographic methods for sex and age estimation to add to the existing forensic anthropological literature will also be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an alternate combined approach for objective and reliable age estimation from dental remains, in addition to existing dental, anthropological, and biochemical methods. Both the techniques are relatively cheaper and faster, delivering comparatively reliable results with higher accuracy rates.

Teeth generally serve as repository of valuable biological information about an individual, and they can reveal information about biological identity, residential mobility patterns, dietary habits, growth and development, exposure to disease and pollutants, social and geographical status of an individual or population groups, etc.<sup>1,2</sup> Trace elements usually get deposited in human hard tissues upon their prolonged consumption in dietary intakes, which, in turn, can decode lifetime events and activities of an individual to facilitate their identification. The elemental composition of human teeth has been widely studied from medical, public health, and dietetics perspectives, but has rarely been explored for forensic anthropological purposes. The requirements and preferences of food items vary with sex, age, disease, health, and geographical status of an individual. The amount of trace elements deposited in the human hard tissues can be used as a reliable indicator of their biological identity.<sup>3,4</sup>

The goal of the present investigation is to characterize the elemental profile of contemporary human teeth from some northwest Indian states and apply the discriminant functions and regression equations developed from known teeth samples to the elemental profile of unknown ancient teeth excavated from an abandoned well situated underneath a religious structure at Ajnala, Amritsar, India. Thousands of unknown human skeletal remains (along with some contextual items) were excavated non-scientifically by amateur archaeologists from the well in early 2014, after obtaining clues from some written records in the form of a book.<sup>5,6</sup> The identity of these remains posed a serious challenge for the investigators because of their badly damaged and seriously commingled nature. Hence, multitudes of anthropological, biochemical, radiological, and molecular techniques were used with the remains to arrive at their valid objective identifications. The main objective of the present study was to reconstruct the probable biological age of the remains from elemental and radiographic analysis of known molars collected from contemporary individuals and applying the developed customized logistic regression and discriminant formulas. Four hundred eighty human molar teeth (230 contemporary and 250 ancient teeth) were used for their elemental composition using a Wavelength Dispersive X-Ray Fluorescence (WD-XRF) analyzer and 703 canines were used for radiographic Pulp-Tooth area Ratio (PTR) estimation from their apical images using Gimp software. PTR was calculated by dividing the ratio of tooth pulp chamber by its outer surface area marked using apical radiographic images of the tooth. Sex prediction was performed using tooth measurements and customized logistic regression formulas for nearly every maxilla/mandible sample. Age estimates of the remains ranged between approximately 20–50 years using both the elemental and radiographical methods. These results corroborated other methods of age estimation, including skeletal anthropological techniques and biochemical analysis. Sex assessment from odontometrics and PTR showed that approximately 90% of the samples were categorized as males.<sup>7</sup> This potentially endorsed the written record theory that these remains do indeed belong to the 1857 conflict.<sup>8</sup>

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### Forensic Anthropology, Age Estimation, WD-XRF and Pulp-Tooth Area Ratio

## G18 Magnetic Resonance Imaging (MRI) of Third Molars, Clavicles, and Wrists for Age Estimation: A Combined Bayesian Approach

Jannick De Tobel, MD\*, Ghent University, Department of Radiology, Gent, Oost-Vlaanderen 9000, BELGIUM; Elke Hillewig, MSc, Universiteit Gent, Vakgroep Radiologie, Gent 9000, BELGIUM; Ines Phlypo, DDS, Universiteit Gent, Vakgroep Tandheelkunde, Gent 9000, BELGIUM; Michiel B. de Haas, BSc, Netherlands Forensic Institute, The Hague 2497 GB, NETHERLANDS; Mayonne Van Wijk, MSc, Netherlands Forensic Institute, The Hague 2497 GB, NETHERLANDS; Steffen Fieuws, Leuven, VB, BELGIUM; Koenraad L. Verstraete, PhD, Universiteit Gent, Vakgroep Radiologie, Gent 9000, BELGIUM; Patrick W. Thevissen, PhD, KULeuven, Leuven, Vlaams-Brabant B-3000, BELGIUM

**Learning Overview:** After attending this presentation, attendees will understand how combining MRI of third molars, clavicles, and wrist for age estimation leads to a sounder age estimation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by elaborating on the emerging field of radiation-free age estimation by means of MRI. It will be demonstrated that age estimation performance increases when combined information is used, provided that an appropriate statistical approach is applied.

**Background:** Currently, forensic age estimation in living adolescents and subadults is based on radiographs and Computed Tomography (CT) imaging.<sup>1,2</sup> These techniques imply an exposure to ionizing radiation outside a medical diagnostic or therapeutic indication. Therefore, their use is prohibited in age estimation cases in several countries. Moreover, these techniques have mostly been studied applied to one anatomical structure at a time in a retrospective design. Unfortunately, to this day, we do not know how the development of different anatomical structures correlate to each other. Consequently, we do not know how to appropriately combine results from studies focusing on one structure at a time.

To counter these drawbacks of the current approach, several research groups are studying the use of MRI of different anatomical structures for age estimation in living adolescents and subadults. So far, only one group has published results of a combined MRI approach, including third molars, both clavicles, and the left wrist.<sup>3</sup> Based on deep convolutional neural networks, they obtained a mean absolute error of 1.14 years (standard deviation 0.96 years). They defined the proportion of correctly identified minors as sensitivity, which equaled 88.6%. However, they did not report how the development of the different anatomical structures correlated to each other.

**Purpose:** (1) To introduce a Bayesian approach for age estimation based on MRI of third molars, both clavicles, and the left wrist; (2) to study its age estimation performance and its ability to discern minors from adults; and (3) to study how the development of the different anatomical structures correlates to each other.

**Materials and Methods:** According to the Ghent protocols, 3-Tesla MRI was conducted of all third molars, both clavicles, and the left wrist in 302 healthy Caucasian volunteers (160 females, 142 males) between 14 and 26 years of age.<sup>4-6</sup> Images were then assessed, applying an optimal staging technique for the development of these anatomical structures, as suggested in previous papers.<sup>5,7,8</sup> Several human observers assessed the images to study reproducibility. Consequently, the data were imported into the Leuven validated Bayesian ad hoc procedure for age estimation.<sup>9</sup> This allowed defining the correlation between the different anatomical structures' development. Moreover, the Bayesian model allowed comparing age estimation performance when different combinations of anatomical structures were included in the model. Accuracy of the models was compared by calculating the Mean Absolute Errors (MAE) and Root Mean Squared Errors (RMSE). Furthermore, the width of confidence intervals and their coverage were calculated, as was the ability to discern minors from adults by comparing diagnostic statistics (sensitivity, specificity, and discrimination slope) and calculating probabilities to have reached the 18-year threshold.

**Hypothesis:** Combining the MRI information of all third molars, both clavicles, and the left wrist will render a better age estimation performance than the separate approaches in living adolescents and subadults. Since this is ongoing research, there were no results available at the time this abstract was submitted to confirm or reject the hypothesis.

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### **Age Estimation, Combined Dental and Skeletal, Magnetic Resonance Imaging**



## G19 The Use of Cone Beam Computed Tomography (CBCT) to Examine the Relationship Between Mandibular Bone Density and the Disappearance of the Clarity of Root Pulp Visibility (RPV) and Periodontal Ligament Visibility (PLV)

Victoria S. Lucas, PhD\*, King's College London Dental Institute, London SE1 9RT, UNITED KINGDOM; Fraser McDonald, PhD, King's College London, London SE1 9RT, UNITED KINGDOM; Graham J. Roberts, MDS, King's College London, London SE1 9RT, UNITED KINGDOM; Mark D. Viner, MSc, Cranfield Forensic Institute, Shrivenham, Wiltshire SN6 8LA, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will be aware of the potential for CBCT to measure the dimension of bone buccal to the roots of the mature lower third molar. Attendees will be able to understand how the increase in the dimension of bone buccal to the mature third molar contributes to the gradual obliteration of both RPV and PLV.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to increased precision when estimating the probability that a subject is over 18 years old.

**Introduction:** The loss of visibility of RPV and PLV related to lower mature third molars (Demirjian Stage H) has been reported by European workers with more recent confirmation of their findings.<sup>1-4</sup> Although the Balance of Probability was 50% for civil cases in the United Kingdom, the Immigration Court is now demanding "beyond a reasonable doubt," which is 100% certainty.

It is perceived that the CBCT will offer the opportunity to measure the thickness of dentin and bone leading to loss of RPV and PLV.

**Materials and Methods:** Approval for the study was obtained from Guys and St. Thomas's NHS Trust (IRAS 231512). CBCTs and corresponding Panoramic radiographs (DPTs) were drawn from the radiographic archive. Three points were identified in the sagittal plane at the bifurcation, half way down the root and approximately 1mm up from the root apex. The view was switched to the horizontal plane and, at each of these points, the distance from the buccal pulpal wall to the buccal surface of the bony cortex was measured.

For both RPV and PLV separately, the measurements were averaged to give a single figure for the buccal bone dimensions.

### Results:

There was concordance between the Left and Right sides (Table 1)

Bone Dimension	Right			Left			t	p
	n	$\bar{x}$	sd	n	$\bar{x}$	sd		
RPV	15	8.0	0.88	18	7.85	1.24	0.3928	0.6971
PLV	15	8.7	1.19	18	8.49	1.24	0.1849	0.8545

There is no significant difference in the thickness of buccal bone between the left and right sides of the mandible;  $n=17$  for left side PLV as some measurements contributing to the average could not be made.

There were slight differences between females and males (Table 2)

Bone Dimension	Females			Males			t	p
	n	$\bar{x}$	sd	n	$\bar{x}$	sd		
RPV	16	8.01	1.14	11	8.17	0.97	0.7093	0.3547
PLV	16	8.46	1.14	11	8.09	2.55	-0.5083	0.6157

This indicates a slight trend toward slightly thinner buccal bone in females compared with males for RPV. This trend is reversed for PLV.



**Discussion:** These data confirm that the bone buccal to the lower left third molar has symmetrical growth on both the left and right sides. This is important because it has been suggested that differences in the appearance of the left and right sides of dental panoramic tomographs account for differences in RPV and PLV assessments.<sup>5</sup> It has not been possible to fully resolve this issue because in the present study, there are insufficient DPTs to match with the corresponding CBCTs of individual patients. This would be a specific topic for future work.

**Conclusions:** The important outcome is the strong similarity of the mandibular bone dimensions on the left and right sides.

The CBCT technique has shown great potential for overcoming the limitations of the DPT technique for assessing RPV and PLV of the mature third molar.

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**Root Pulp Visibility (RPV), Periodontal Ligament Visibility, Bone Dimensions**



## G20 UT-Age 2018: An Updated Tool for Third Molar Development Age Assessment

Leigh-Ann Schuerman, DMD\*, Cave Creek, AZ 85327; James M. Lewis, DMD, Madison, AL 35758; David R. Senn, DDS, University of Texas HSC San Antonio, San Antonio, TX 78229-3900

**Learning Overview:** After attending this presentation, attendees will understand the advantages of using the automation, report formatting, and data storage features of a computer program for third molar age assessment cases. Updates to one such program, UT-Age, will be discussed and demonstrated.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the use of a computer program, UT-Age, to standardize, automate, and create databases for third molar age assessment cases. UT-Age 2018 now incorporates censored Stage H data, the ability to calculate probability for varying jurisdictional legal ages of majority, and now permits report editing in Microsoft® Word®.

UT-Age was originally developed in 2002 as a Microsoft® Access®-based application and database. In 2008, UT-Age was updated to a .NET Framework using Microsoft® Access® as the database. In 2018 .NET framework provides a large body of pre-coded solutions to common program requirements and manages the execution of programs written specifically for the Microsoft® operating system. UT-Age 2018 continues the use of .NET Framework and therefore is intended for use on Windows® operating systems.

UT-Age 2018 incorporates many of the same features as previous versions of UT-Age. This computer application archives data for third molar age assessment cases, cataloging the subject's case number, name, ancestry, sex, facial photographs, stated date of birth, and radiograph(s). Based on the user-entered stage of morphologic development of the third molars present, the program calculates the average mean estimated age of the individual, and the average age interval to two standard deviations. Previous versions of UT-Age calculated the average empirical probability of the individual having attained her/his 18<sup>th</sup> birthday only. However, UT-Age 2018 now allows the investigator to select the target age for which empirical probability will be calculated.

These UT-Age features can: (1) assist by providing information to those involved with human trafficking cases, (2) provide information to those charged with helping to protect juveniles in custody from being incarcerated with adults (or adults with juveniles), (3) provide information to assist with the defense of criminal and/or civil prosecution of undocumented individuals, and (4) provide information for other cases involving adolescents or young adults (with third molars) regardless of the situational or jurisdictional questioned age.

Publications and presentations, including at the American Academy of Forensic Sciences, have reported the importance of censoring the terminal morphologic developmental stage of the Demirjian staging system (Stage "H"). Censored Stage "H" data for Kasper et al., 2009; Blankenship, Lewis and Senn, 2010; and Mincer et al., 1993, has been recently published.<sup>1</sup> UT-Age 2018 includes updated statistical population data intended to improve the reliability of the reported age estimations for individuals that have third molars that have reached the terminal stage "H."

The UT-Age 2018 application will be available by February 2019 for online download on two websites: [utforensic.org](http://utforensic.org) and [gsm.utmck.edu/dentistry/fellowship.cfm#forensic](http://gsm.utmck.edu/dentistry/fellowship.cfm#forensic).

### Reference(s):

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### Dental Age Assessment, UT-Age, Third Molar



## G21 The Dental Age Research London Information Group (DARLInG) Development of a Database of Dental and Related Data to Enable Logical Dental Age Estimation (DAE)

Graham J. Roberts, MDS\*, King's College London, London SE1 9RT, UNITED KINGDOM; Victoria S. Lucas, PhD, King's College London Dental Institute, London SE1 9RT, UNITED KINGDOM; Fraser McDonald, PhD, King's College London, London SE1 9RT, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will understand the techniques involved in developing a database for dental age estimation, and the advantages of the referential integrity a database confers by ensuring robust data output to Microsoft® Excel® and Stata statistical software. Attendees will see that the integration of the DARLInG database output with Draft Age Estimation Quick Sheets™ completes a program of innovative database and spreadsheet work that enables quick and reliable estimates of age in children, adolescents, and young adults.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through the combination of the DARLInG database and the Draft Age Estimation Quicksheets™ to achieve rapid dental age estimation based on a dental panoramic tomograph.

**Introduction:** Data processing of Ages at Assessment (AaA) of Tooth Development Stages (TDS) becomes unwieldy when several thousand cases are included in the Reference Data Set (RDS). This is the main difficulty limiting numerical data management to Microsoft® Excel®. The Microsoft® Access® database overcomes this problem by enabling the use of specially fabricated forms that enables data entry in a convenient way. There are four components to the Microsoft® Access® database.

**Access Tables:** These form the principal component of the data management process. A single table for personal data is defined within the DARLInG database. This uniquely identifies every subject from whom data are derived. A feature of the database is that all the data are cross-sectional. Thus, bias from canalization does not enter the processing of dental age estimation assessments.<sup>1</sup>

The individual fields for data are defined within the table. This is important as once the data are exported for statistical analysis, the field definition is exported with it. The consequence of this is that a categorical field that defines gender must be created with this in mind, so when exported to a statistical software package, the definition of female or male is immediately apparent. Similarly, with data for ages or measurements, the fields are defined as numerical values so that statistical computations can be carried out appropriately.

Crucial to the importance of the Tables is the use of a Primary Key. In the DARLInG database, this is the field that enables the data from subjects to be uniquely and precisely linked. The field name used is "idno" (Identity Number). When data for a new subject is entered, the Primary Key definition generates a random number from -1,00,000,000 to +1,000,000,000. This ensures that the idno cannot be duplicated and is irrevocably linked to all the data for a single case. This is the principle of Referential Integrity.

**Access Forms:** These are created using the data fields present in the tables related to the database. The use of forms enables the 128 fields from the Demirjian TDS to be presented as a box matrix on one single computer screen. Thus, accurate and quick data entry are possible.

**Access Queries:** This is held in the databases and is extracted to enable meaningful data sets to be created. For example, the gender and age of a subject from one table, TDS from a separate table, and perhaps Stages of Development of the wrist bones from a third table. All of these are tightly linked by the idno, which is the primary key.

**Access Reports:** Data selected from the database are presented in an easily comprehended form. The DARLInG database does not use Reports, as the queries are exported to Microsoft® Excel® because the exported data is linked into Draft Age Estimation Quick Sheets™.

This serendipitous collaboration means that the two software-developed procedures can be used to provide immediate and reliable estimates of dental age.

To date, there are 15,411 cases in the DARLInG database utilizing information from data on Demirjian, Haavikko, and Moorrees TDS, Cervico Vertebral Maturation, root canal width, root pulp visibility, and periodontal ligament visibility, and data from hand wrist X-rays, sterno-clavicular joint radiographs, and measurements from cone beam computed tomographs.

There are Demirjian TDS data from five different ancestral groups.<sup>2</sup> This enables comparison of DAE assessments using *exactly* the same methodology.

### Reference(s):

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<sup>2</sup>. <http://www.dentalage.co.uk/+R> (to access Reference Data Sets)

### DARLInG, DAE Database, Dental Age Estimation



## G22 Using Photoshop® for Odontology Doesn't Have to Bite

David Witzke, BA\*, San Diego, CA 92103

**Learning Overview:** After attending this presentation, attendees will understand digital imaging concepts and how they should be applied in a forensic environment. Attendees will also gain a basic understanding of the tools available in Adobe® Photoshop®, as well as how to apply them when creating images for analysis and comparison, and how to discuss the processes in the courtroom.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the basic concepts and guidelines of digital imaging and how to ensure that those concepts are applied properly so another innocent person is not sent to jail because someone was using technology improperly.

In 1979, the Connecticut State Supreme Court was the first court to adopt ground rules for the admissibility of computer-enhanced evidence to safeguard the right of defendants to challenge its reliability in accordance with *Federal Rules of Evidence* 901.<sup>2</sup> In its 1979 ruling in *American Oil Co. v. Valenti*, the court stated that there must be "... testimony by a person with some degree of computer expertise, who has sufficient knowledge to be examined and cross-examined about the functioning of a computer."<sup>3</sup> It further stated that evidence presented using state-of-the-art computer technology must be accompanied by an expert's explanation of how the process works and must allow for cross-examination on whether the evidence was in any way manipulated or altered. The court also adopted the guidelines regarding admissibility of evidence presented via computer-based technology set forth in *Federal Rules of Evidence* 901.<sup>2</sup>

In a 2004 case, *State of Connecticut v. Alfred Swinton*, the Connecticut Supreme Court reiterated the rule that there must be "testimony by a person with some degree of computer expertise, who has sufficient knowledge to be examined and cross-examined about the functioning of the computer" but also took the opportunity to clarify its 1979 position on the guidelines set forth in *Federal Rules of Evidence* 901.<sup>4</sup>

In the Swinton case, the odontologist could not answer basic questions about the technology; he could not explain the process used for creating the overlays; and he could not answer whether Adobe® Photoshop® was used in the field by other odontologists. Yet Alfred Swinton was found guilty based upon the presentation of evidence (using an overlay created in Adobe® Photoshop®).<sup>5</sup>

During the trial, the odontologist testified with "a reasonable medical certainty without any reservation" that the bitemarks on the victim were created by Alfred Swinton. However, in an affidavit submitted as part of Swinton's petition for a new trial, the odontologist recanted his trial testimony. The odontologist wrote: "I no longer believe with reasonable medical certainty—or with any degree of certainty—that the marks on Ms. Terry were created by Mr. Swinton's teeth, because of the recent developments in the scientific understanding of bitemark analysis." After 19 years in prison, Alfred Swinton was exonerated and released from prison.

The information, technology, and techniques demonstrated in this presentation will provide answers to the questions raised in *Federal Rules of Evidence* 901 (also known as the "Swinton Six").<sup>6,7</sup>

This presentation will reveal how resolution effects image quality and image size, which influences how images are displayed or printed. It will also show how these digital imaging concepts and guidelines ensure the highest level of accuracy and detail when capturing digital images of dental impressions and bitemarks.<sup>8</sup>

Without this knowledge, it is impossible to create overlays properly for detailed analysis (annotation), comparison, and evaluation. It is equally important to recognize the loss of information (degradation of image quality) that can occur when certain file formats are used to store digital images. Together, these concepts and guidelines play a significant role in the scientific understanding of bitemark analysis and comparison.

### Reference(s):

1. *Federal Rules of Evidence (2018 Edition)*. Article IX, Rule 901, Authenticating or Identifying Evidence.
2. *State of Connecticut v. Alfred Swinton*, 847 A.2d 921 (Conn. 2004).
3. *American Oil Co. v. Valenti*, 179 Conn. 349, 359, 426 A.2 d 305, 310.
4. Guthrie, Catherine & Mitchell, Brittan. *The Swinton Six: The Impact of State v. Swinton on the Authentication of Digital Images*, 36 Stetson Law Review 661 (September 26, 2007).
5. McDonough, Molly. *Enhancing Rules for Digital Data: Connecticut's Top Court Lays Down the Law on Computer-Modified Evidence*, 3 (21) ABA J. E-Report (May 28, 2004).
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### Digital Imaging, Forensic Imaging, Photoshop®



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## G23 Digital Image Processing for the Forensic Dentist

*James McGivney, DMD\*, St. Louis, MO 63119*

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**Learning Overview:** After attending this presentation, attendees will: (1) be able to differentiate between malicious and non-malicious image processing; (2) be aware of the existence and availability of several computer apps used to change digital graphics; and (3) know how to crop, resize, and change the format of digital images.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing forensic dentists to be aware of how digital images can be manipulated.

Digital images are captured, viewed, manipulated, shared, and stored by forensic dentists. Computers and digital records encompass two distinct fields in forensics. Computer forensics, also known as computer forensic science, is a branch of forensic science pertaining to evidence found in computers and digital storage media, while forensic photography, also referred to as crime scene photography, is an activity that records the initial appearance of the crime scene and physical evidence as an aid in investigation and to provide a permanent record for use in the courts and the legal system.

The forensic dentist needs to be aware of the difference between malicious and non-malicious image processing. The purpose of malicious image processing is to deceive the viewer. In dental radiography, caries, fracture, and other pathology can be added to a digital image to aid in obtaining reimbursement from a third-party payer.

Non-malicious image processing makes a graphic easier to view and may highlight the detail in a specific portion of the graphic. The cropping of an image allows the eyes to focus on the subject, while eliminating extraneous elements. Adjusting the brightness, contrast, and color balance of a poorly exposed photograph can yield a more pleasing and informative image.

The marking detail of individual teeth in a bitemark graphic can, at times, be enhanced by adjustment of the color levels. This type of image processing is known as feature extraction and is intended to facilitate human interpretation.

In bitemark images, four distinct types of distortion have been identified, some of which can be corrected by image processing.

The processing of an image leaves tell-tale signs. The detection of image processing relies on various techniques and is known as recognizing if a graphic has been “photoshopped.” The presence of signs of image processing can range from very subtle to quite evident. Shadows and reflections should be realistic in location, size, and depth. Skin color should not appear too perfect or polished. Round selection tools may leave obvious circular patterns in the manipulated graphic. Areas that show excessive blurring and the presence of irregular or broken lines in patterns also reveal evidence of image manipulation.

The working knowledge of the use of several computer applications will benefit the forensic dentist. These applications include the GIMP image manipulation program, Windows® Snip® tool, and Google’s® reverse image search. These apps are available online at no charge. This presentation will guide the forensic dentist in the utilization of these apps to process images and to detect the presence of image manipulation.

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**Digital, Image, Forensic**



## G24 Improvements to Digitized Radiographic Images Using Adobe® Photoshop®

*Robert E. Wood, DDS, PhD\*, Ontario Forensic Pathology Service, Toronto, ON M5G 2M9, CANADA; David Sweet, OC, DMD, PhD, BOLD Forensic Lab at UBC, Vancouver, BC V6T 1Z3, CANADA*

**Learning Overview:** After attending this presentation, attendees will have improved their understanding of: (1) problems that exist when faced with underexposed or overexposed radiographs in comparative dental identifications; (2) methods to use a raster graphics editor to change exposure factors of problematic radiographic images; and (3) simple, straightforward steps that can be taken to adjust the radiodensity of images.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing attendees' understanding of the issues and provide methods to improve their approaches to routine dental identification casework (increased performance).

It is believed that most odontologists are faced routinely with analogue intraoral radiographs within supplied antemortem records from missing persons that are not optimal for use in comparative dental identification cases because of image mode, exposure, and radiodensity limitations.

This presentation will discuss problematic issues that the forensic odontologist often faces when responding to requests for forensic physical comparison of dental records produced on unidentified found human remains to dental records of a missing person. Typically, because of available circumstantial evidence, death investigators have already established a putative identification of the human remains. For example, when a wallet is found among the possessions on or near the body or the decedent is the driver of a registered motor vehicle with a license plate and vehicle registration number. This putative identification then must be confirmed through reliable methods. Based on the investigative leads and this putative identification, a search is conducted for the person's Antemortem (AM) dental records.

Postmortem (PM) dental records are produced by the odontologist at the time of the dental examination during the dental autopsy. Quality control measures are used by the odontologist to determine if the PM records meet the standard of care for living persons by the person's dentist of record. This will ensure that the interpretation of data from the PM records can be directly compared to the data present in the AM records from the missing person. If the PM dental records (e.g., PM radiographs) are determined to be suboptimal, the attending odontologist can re-take them to obtain high-quality data that will enable direct comparisons to any available AM records.

The quality of AM dental radiographs obtained from the missing person's dentist of record may be suboptimal, with variations in radiodensity and exposure factors, processing errors, etc. Of particular concern are analog, film-based AM radiographs that are supplied and are the only ones available. These AM radiographs may be original films, digital images, or scanned images produced from the original films. There are various reasons why the dentist of record may not have been able to produce high-quality AM films. A patient's fear of radiation exposure; tolerance of closing the mouth to hold the image receptor or film packet adequately; the over- or underexposure of the image by the X-ray generator from mistakes or misunderstanding of the dental assistant; and improper development, fixation, or washing of the film prior to long-term storage are but a few examples of how less-than-ideal AM radiographs may be present in a submitted dental record for any missing person. Further problems arise when film-based images are copied for submission into the death investigation system since copy-film does not contain information regarding directionality. How can the odontologist maximize the ability to interpret adequately what is recorded in such problematic radiographs?

Methods are available to the odontologist to increase the ability to review, find, and employ various key dental traits useful in forensic comparisons. These methods enhance the ability for the odontologist to visualize data that is present but is hidden or masked by the problem factors previously mentioned. By making corrective adjustments to problematic AM films or scanned images, meaningful comparisons may still be possible.

Adobe® Photoshop® is a raster graphics and image editor that is used on Windows® and MacOS® computers for imaging processing. This computer application has become so popular that it is considered a generic trademark and the word "photoshop" is often used as a verb when one is describing the process of adjusting an image. Methods utilizing Photoshop® will be demonstrated during this presentation to allow attendees to appreciate the dramatic and significant changes that can be achieved with simple steps to change certain images factors, including but not limited to color versus grayscale image modes, exposure factors to change the tonal range, and color balance of an image (i.e., levels adjustment), etc. A handout will be available in PDF format that will summarize these steps.

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### Odontology, Dental Identification, Image



## G25 Digital Techniques for Dento-Facial Superimposition

John B. Nase, DDS\*, Dental Forensic Services of Indian Valley, Harleysville, PA 19438

**Learning Overview:** After attending this presentation, attendees will be able to outline a method of utilizing postmortem and antemortem victim portraits with Adobe® Photoshop® CS and other software to generate both still and sequential “movie” comparisons of the visible smile. Attendees will also be able to appreciate how computer techniques learned in bitemark comparison and age estimation can be repurposed for dento-facial superimposition. A case report using this method that supported a positive victim identification will be presented. Current limitations and recommendations will also be discussed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing an alternate dental method via digital images to support identification of a presumed victim when traditional antemortem dental records do not exist or are not recovered.

In March 2018, a decedent was recovered from the water. The probable victim’s identification was presumed from circumstances of a one-week missing 72-year-old woman with Alzheimer’s disease from northeast Philadelphia, PA. Although there were no existing antemortem dental records for the presumed victim, her anterior dentition was reported as “distinctive,” and the family had supplied several family photographs exhibiting her smile. The medical examiner asked to perform a photographic dento-facial superimposition to corroborate other findings.<sup>1</sup>

As instructed, included in the autopsy were several digital images of the decedent’s face, to include lip retraction and a two-legged, photomacrographic #2 scale, from several angles. The postmortem image with the closest matching plane of view was superimposed onto one of the digitized antemortem images, utilizing digitally constructed hollow volume and solid volume exemplars using Photoshop® CS. All images used for overlay comparisons were individually scaled to a 1:1 ratio, first using the photometric scale in the postmortem image, then scaling the antemortem image by using the maximum width of the left central incisor as the standard in both. Metric analysis of anatomical landmarks, using the corrected image scale, was also performed as appropriate. Comparison of the maxillary and mandibular portions of the known dentition to the hollow and solid volume exemplars of the presumed dentition were analyzed for concordance with respect to arch width; the morphology, positions, angles, and spacing of individual teeth; and incisal edge topography.<sup>2,3</sup> As a visual aid to presenting the comparative findings, the images in the Adobe® Photoshop® CS workspace were then exported to Techsmith Camtasia software to create a sequential video file.

The unidentified decedent demonstrated individual arch and tooth characteristics that were consistent with the dentition of the presumed individual, with no unexplainable discrepancies. Therefore, the visible dentition of the presumed individual could NOT be excluded as being the same as the decedent. This conclusion supported the medical examiner’s corroboration of findings in a positive identification.

In conclusion, this case shows utilization of digital tools and techniques, borrowed from other odontological disciplines, in the dento-facial superimposition of strategically produced postmortem coronal head images to family photographs to corroborate unknown victim identification.

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### Superimposition, Photoshop®, Comparison



### G26 Combining 3-Dimensional (3D) Technology With the Traditional 2-Dimensional (2D) Smiling Photographs to Aid in Accurate Forensic Dental Identification

*Gowri V. Reesu, MSc\*, University of Dundee, Dundee, Scotland DD1 4HR, UNITED KINGDOM; Scheila Manica, PhD, University of Dundee, Dundee, Scotland DD1 4HR, UNITED KINGDOM; Gavin F. Revie, PhD, University of Dundee School of Dentistry, Dundee, Scotland DD1 4HR, UNITED KINGDOM; Peter A. Mossey, PhD, University of Dundee School of Dentistry, Dundee, Scotland DD1 4HR, UNITED KINGDOM*

**Learning Overview:** After attending this presentation, attendees will better understand the application of 3D technology in accurate forensic dental identification using smiling photographs and will appreciate the difference in certainty of conclusions reached with other comparison methods. Additionally, attendees will learn the advantages of using 3D software in the field of forensic odontology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by exploring a novel technique for increasing the accuracy in dental identification, which supports further research in the field.

**Background:** Accurate human identification is of utmost importance for humanitarian, legal, and social reasons. The methodology of comparative identification is based on comparison of Antemortem (AM) and Postmortem (PM) records. However, as with many methods used in human identification, traditional forensic odontologic techniques can at times be unsuitable for reasons including lack of dental records and when information from available dental records is irrelevant to investigation. Smile photographs may emerge as valuable alternative sources of AM data in the forensic environment. They specifically register the unique features of the anterior dentition while recording the shape, position, angulation, incisal alignment, and occlusal relation of visible maxillary and mandibular teeth.

Technological advances in 3D imaging enable useful techniques for the interpretation of smile photographs with human identification purposes. Facilities such as 3D surface laser-imaging technologies have spawned new applications in dentistry. Using a laser scanner, dental plaster models can be converted into 3D images that may be viewed in any preferred orientation and may be subjected to quantitative analysis.

**Goal:** To explore novel odontological methods by comparing 2D digital smiling photographs with 3D dental models.

**Materials and Methods:** The study sample consisted of 35 randomly selected smiling photographs of patients who gave consent to participate in research in the Orthodontics Department, Dundee Dental Hospital, Dundee, Scotland. The photographs of the subjects obtained for this study were of varying quality. The smiling portion in the images were cropped and archived, and these images were considered as AM images.

Thirty-one dental casts of the same photographic sample were retrieved from the orthodontic laboratory, which were archived for treatment purposes. All the dental casts were laser scanned to create indirect 3D digital images of dental models and were considered as PM digital models. This study was conducted in two phases. In phase one, 31 digital 3D models were visually compared with 35 of the smile photographic samples showing upper and lower front teeth (canine to canine). The opinions were reached based on the International Criminal Police Organization (INTERPOL) Disaster Victim Identification (DVI) guidelines: Identity Established, Probable, Possible, and Exclusion. After a wash-out period of a week, the second phase of comparison was made using 3D Rhinoceros® software in which the selected 2D AM photograph was superimposed with the 3D digital model, with emphasis on incisal contours of anterior teeth and any morphological traits. Both methods of comparison were performed by the principal investigator.

This study hypothesizes that a valid imaging technique will aid in increasing the accuracy of human dental identification by comparing smiling photographs with dental models. The justification is based on shape, dimensions, and alignment of teeth of an individual, which can comprise a specific and unique set.

**Results:** Phase 1—In direct comparison of AM photographs and PM dental models, conclusions were reached in 26 cases (identity possible: 16; probable: six and four as established) only; one was wrongly identified, and it was not possible to form an opinion in four cases. Phase 2—With the application of 3D comparison software, conclusions were reached in all the 31 samples (identity possible: seven; probable: 13 and 11 as established) and were correct. This 3D technique made it possible to reach opinions in more cases than when compared to direct comparison. A significant increase in certainty among the opinions reached was observed when using the 3D comparison software.

In summary, it was possible to reach a conclusion in more cases when using the 3D comparison software, and those conclusions expressed a higher degree of certainty, which is the crux of this study as to whether the 3D comparison software adds significant value.

#### Forensic Dental Identification, Smile Photographs, 3D Comparison

## G27 The Prevalence of Morphological Variations of the Maxillary Sinus in Panoramic Radiographs of Caucasian Individuals

Ludovica Gorza, MSc\*, Padova 35125, ITALY; Patrick W. Thevissen, PhD, KULeuven, Leuven, Vlaams-Brabant B-3000, BELGIUM

**Learning Overview:** After attending this presentation, attendees will understand morphologic classifications of the maxillary sinuses and the surrounding morphological features observed in panoramic radiographs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insights into the frequency and variability of anatomic parameters of the maxillary sinuses observed in panoramic radiographs and will allow quantifying of their identifying performances.

**Introduction:** In contemporary comparative forensic dental identification practice, the collected Antemortem (AM) evidence contains mainly descriptive registrations and 2D radiographic images. Therefore, certain anatomical characteristics of the maxillary sinus that are visible in 2D radiographs can contribute to the positive identification of unknown individuals.<sup>1,2</sup>

**Purpose:** The goal of this study was to classify specific morphologic parameters of the maxillary sinuses on panoramic radiographs and to quantify their variability for human identification purposes.

**Materials and Methods:** Retrospectively, 500 digital panoramic radiographs from Caucasian male (250) and female (250) individuals between 10 and 70 years old were collected. The following parameters of the maxillary sinuses were analyzed: unilateral or bilateral presence; the peripheral shape; the symmetry of the peripheral contours; the geometry of the medial walls; the unilateral or bilateral visibility of the infraorbital canal, the pterygomaxillary fissure and the infraorbital ethmoidal air cells; the shape of the innominate line of the zygomatic process and the antral septa; the topographic relation between the roots of posterior teeth and the sinus floor; and the presence of alterations in the mucous membrane and cortical bone. Moreover, the maximal height, maximal length, and the area and distance between the right and left sinuses were also measured using GIMP 2.10.4 for Windows®. For inter- and intra-observer reliability tests, randomly 40% of the radiographs were re-assessed and analyzed with Kappa statistics. Analysis of Variance (ANOVA) statistics were applied to quantify the variability of each parameter and to classify them in function of their identifying ability. The analyses were performed sex-specific and compared between left and right maxillary sinus.

**Results:** Currently, not all collected data has been analyzed, but intermediate results and this study hypotheses indicate that: (1) there is a significant prevalence of present maxillary sinuses; (2) the overall peripheral shape of the maxillary sinus has significant variability, with asymmetrical sinuses being more prevalent; (3) the medial walls have mainly oblique medial orientations; (4) there is a high prevalence of visibility of the infraorbital canal, the pterygomaxillary fissure, and the innominate line of the zygomatic process; (5) there is a low prevalence of infraorbital ethmoidal air cells and alterations in the mucous membrane and the cortical bone; (6) the prevalence of antral septa is statistically significant in edentulous or partially edentulous maxillae compared to dentate maxilla; (7) the prevalence of roots projecting in the maxillary sinus is significant; and (8) there is no statistically significant difference in the measures between female and male and right and left sinuses. Certain morphologic parameters of the maxillary sinuses observed in panoramic radiographs can be considered as additional odontological identifiers.<sup>3-6</sup>

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**Forensic Odontology, Human Dental Identification, Radiographic Maxillary Sinus**



**G28 WITHDRAWN**



**G29 WITHDRAWN**



### G30 When Arson Becomes Murder: Antemortem Records Fire the Identification Process

*Randolph L. Mitchell, DMD\*, Lyons, NY 14489*

**Learning Overview:** After attending this presentation, attendees will have learned to await the development of all possible leads for antemortem dental records after a death that has rendered the victim unidentifiable by conventional means. Attendees will learn to have patience as the investigation of the death continues and to avoid the temptation of trying to make a positive identification without adequate records to support the identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by opening the minds of the investigative team when searching for antemortem information by increasing their awareness of some unusual local sources of this information at the scene. This information can narrow their records search to a much more focused area, speeding the acquisition of the antemortem information and making the positive identification possible.

Often, when the decedent is found in a location where there was no known person living and with no personal effects available, the investigators seem to be at a dead end right from the beginning and must be creative in their investigative techniques to find a logical starting point to determine a “believed to be.” Acquiring the necessary Antemortem (AM) records needed to make a solid dental identification is also very challenging in these cases.

This is perfectly illustrated in an actual case study of an arson case in which the decedent was found with no real identifiers for the investigators to use. The body was literally unrecognizable, burned to the point where there was no intact skin on the body for fingerprinting to be used or even an idea of who to match DNA with. The postmortem dental examination and charting was fairly routine. Even though several of the dental structures were damaged by the fire to the extent that they would be useless for identification purposes, there were several teeth intact that had dental restorations that could be used as excellent exemplars in a dental identification, given some records with which to compare them. There was absolutely no logical place to start the search for AM dental records.

The investigators caught a real break after several days that began to clear the picture of a possible lead on who the victim of this arson was. Some of the local inhabitants of the area knew that someone had been living in the structure that was burned, but since they were homeless and only knew each other by “street names,” more research would be necessary. Eventually, through some information garnered by the investigators from a governmental agency, a possible name for the John Doe was offered. Since the decedent was believed to be homeless, the search for dental records spread outward from the center of a circle defined by the fire scene. Clinics that provide health service to patients who are on social service benefits were checked to see if they had provided services to someone of that name. Dental records were located for a person of that name at one of the local clinics; those records were compared to that of John Doe and yielded a positive identification of the decedent. Once again, the importance of the AM records in making a dental identification is driven home by this case.

#### Sources, Records, Identification

### G31 The Mystery of the Unidentified Human Remains Found Wrapped in Dental Packing Tape and Its Dental Biography

Francesca Tarantino, MD\*, Bari 70124, ITALY; Maricla Marrone, MD\*, Bari 70124, ITALY; Stefania Lonero Baldassarra, Bari 70124, ITALY; Maria M. Lepore, Bari 70124, ITALY; Emilio Nuzzolese, PhD\*, Dental Team DVI Italia, Bari 70124, ITALY

**Learning Overview:** After attending this presentation, attendees will be able to evaluate the application of best practice when performing the identification of unidentified human remains. In the field of missing and unidentified cadavers, it is essential to reach a preliminary generic profile of the unidentified body. This can be obtained only through a multidisciplinary postmortem assessment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the results of an identification casework in which the postmortem collection involved a forensic pathologist, a forensic odontologist, and a forensic biologist, who were able to define the profile of the unidentified person.

In Apulia, one of the 20 regions of Italy with a total of more than four million inhabitants, there are to date 3,548 reported missing persons and 59 unidentified human remains, as highlighted by the Missing Person Association “Penelope Puglia.” In May 2017, an unidentified skeletonized body was found wrapped in dental packaging tape in an abandoned factory in Bari, the capital of the region. The medical examiner appointed by the court was authorized to involve a biologist and a forensic odontologist for a complete postmortem assessment. The anthropological evaluation and the dental autopsy allowed the definition of the generic profile with a specific dental biography, including a dental age estimation using periapical X-ray of the upper and inferior canines. The dental biography was that of a female, aged 55 ( $\pm 5$  years) years old, partially edentulous with only front teeth and one inferior molar, orally neglected, wearing two mobile partial prosthetics, with a high horizontal alveolar bone reabsorption, which resulted in the appearance of long “jagged teeth.”<sup>1,2</sup> The radiological analysis of the jaws revealed infrabony pockets with plausible teeth mobility. The observation of the two partial prosthetics led to the conclusion that they were manufactured seven to eight years previously. Furthermore, the only amalgam restoration was assessed clinically as having been performed approximately five to seven years prior. Dental findings were also recorded in the International Criminal Police Organization (INTERPOL) Unidentified Missing Person form, as the initial nationality of the deceased was not known. A biological DNA sample was collected from the inferior second molar. The condition of the cadaver did not allow for the collection of fingerprints.

Histological findings and a skull Computed Tomography (CT) scan allowed the medical examiner to determine the cause of death as a hemorrhagic stroke, due to several beatings and physical abuse. The forensic dental examination enabled the narrowing of the search to four reported female missing persons, on which the police held some antemortem data. One had been examined for sexual abuse in 2009 and had received a vaginal swab. DNA analysis was performed on this specimen revealing two profiles, one of the victim’s and one of a male abuser. The comparison with the postmortem DNA profile matched with the missing person and all other data was compatible.

This case report confirms the need for always performing a complete dental autopsy in all unidentified human remains cases to reach a speedy identification by narrowing the search of the compatible reported missing persons.

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#### Identification, Missing Persons, Dental Autopsy



### G32 Dental Identification of Carbonized Victims in a 2015 Traffic Accident in France

*Gwenola Drogou, DDS\*, Ploemeur 56270, FRANCE; Charles E. Georget, PhD\*, Amboise 37400, FRANCE; Aime Conigliaro, MSc\*, IRCGN, Pontoise 95300, FRANCE*

**Learning Overview:** After attending this presentation, attendees will understand the need for strict protocol implementation before intervening at a disaster site. Attendees will also understand the importance of forensic odontologists being part of the recovery team, especially in cases involving carbonization.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the forensic odontologist's role is not only to collect antemortem and postmortem indices, but that dental protocols are an integral part of the overall process involving the work of other disciplines to obtain an optimized and effective outcome.

A traffic accident occurred in Puisseguin, France, on October 23, 2015, and caused 43 casualties. This accident was the deadliest event in France since the Beaune accident in 1982, which caused 53 deaths, including 46 children. In the Puisseguin case, two vehicles and 51 persons were involved in the accident. A coach with 47 seniors, their driver, and their tour guide began a sightseeing trip. A trailer truck was moving into the left lane during a turn. It was driven by a 31-year-old driver accompanied by his 3-year-old son. These two vehicles caught fire immediately at the moment of collision, causing 43 casualties and injuring eight people, four of them severely.

According to the Land Transports Accidents Bureau of Investigation, once the fire had started in the bus, the Anti-Breaking System (ABS; thermoplastic polymer), polypropylene, polyester, and polyurethane that made up the interior trim of the vehicle began to burn and release highly toxic fumes, which spread very rapidly throughout the vehicle. Within one hour following the accident, L'Institut de Recherche Criminelle de la Gendarmerie Nationale (IRCGN) (The Criminal Research Institute of the French Gendarmerie) was called to the scene to handle this disaster.

A multidisciplinary postmortem team was deployed on the ground to recover the charred victims. Extreme care had to be taken when moving the bodies, placing them in body bags, and then transporting them to the medicolegal institute in Bordeaux as these were all technique-sensitive processes that could have resulted in the loss of critical forensic materials. Photographs were taken to preserve dental data before moving the corpses. All of the heads exhibited a severe degree of carbonization; front, right, and left lateral photographs were taken as a safeguard measure to document as much evidence as possible. This protocol was complemented by placing special bags around the heads to avoid losing any evidence during transportation. Any carbonized remains that had separated from the victims' bodies were sieved to recover any remaining items that may have been forensically interesting.

One forensic dentist from this PM team was sent to the medicolegal institute in Bordeaux to take part in autopsies, dental examinations, and data collecting. An antemortem team was set up at the Criminal Research Institute of the French Gendarmerie in Pontoise near Paris. One forensic pathologist and forensic odontologists got in touch with victims' families to obtain the maximum amount of antemortem data and all the victims were identified when medical and dental data were sufficient. A small antemortem team was also set up in a classroom in a village close to Puisseguin, where families could come to bring in any medical files and X-rays.

This presentation relates the causes and all dental identification techniques established to identify these traffic accident victims. This presentation demonstrates the importance of the strict observance of the selected protocols and the added value of the forensic odontologists' participation in the recovery of the charred victims.

#### Forensic Odontology, Traffic Disaster, Carbonization



**G33 WITHDRAWN**





### G34 Dental Identification: An Important Thing—The Second Time Around

*Jacqueline S. Reid, DDS\*, Chatham, NJ 07928*

**Learning Overview:** After attending this presentation, attendees will understand the need for dental identification procedures and records maintenance, and how they can assist law enforcement long after the case has been completed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the need for proper identification procedures, report writing, and the long-term maintenance of evidence.

In 2011, Michael Maltese was convicted of killing his parents and burying their bodies in a shallow grave in Beechwood Park, South Brunswick, NJ. In 2015, the Supreme Court of New Jersey affirmed the defendant's convictions for second-degree disturbing, moving, or concealing human remains, fourth-degree tampering with evidence, third-degree hindering apprehension or prosecution, third-degree theft, third-degree fraudulent use of a credit card, and fourth-degree false swearing, and reversed and remanded for retrial the charges of passion provocation manslaughter and first-degree murder.

It was determined that the trial court shall conduct a pretrial hearing to determine whether the physical evidence obtained as a result of the defendant's suppressed statements is admissible under the inevitable discovery exception to the exclusionary rule. Following the inevitable discovery testimonial hearing, the judge ruled that the physical evidence, including the gravesite and autopsy photos were admissible at trial. In 2018, Michael Maltese was retried and found guilty of "passion provocation" manslaughter for both his parents.

The case discussion includes: (1) search techniques for antemortem records; (2) assisting the Medical Examiner (ME) to obtain records; (3) antemortem records review; (4) postmortem examination and data collection; (5) chain of custody; (6) maintenance and digital archiving of dental evidence and case data; (7) report writing techniques; (8) preparing for court; and (9) expert witness testimony .

In October 2008, the ME requested a dental review of photographs of a bitemark on the "pinky" finger of a suspect in custody who confessed to strangling his parents and claimed his father bit him. Upon arrival, the injury photographs provided by the ME were of limited to no value. The ME stated that they could not locate the dental records of the parents and perhaps could look to identify them in some other manner. The suspect/son in custody told the police where the parents were buried. However, it was decided to complete a full dental autopsy, examination, photographs, and radiographs on the parents, nonetheless.

After thoughtful attention and some luck, the search for dental records yielded positive results and a full-mouth series of radiographs for both parents were found. After the comparisons were completed, a positive dental identification was made for Michael and Kathleen Maltese. The dental reports generated were submitted to the ME on November 14, 2008. A subpoena, to be on call to testify, was issued in January 2018. Nearly ten years later, sworn testimony was provided in court on the identification procedures used to determine the identity of the victims found in the grave, at a park in South Brunswick, NJ, on October 24, 2008.

#### **Dental, Identification, Re-Trial**



### G35 A Review of Postmortem Dental Radiographic Protocol for Emergency Responders in Individual to Mass Fatality Operations

*Khlea Sumani, DMD\*, Zanesville, OH 43701; Charles E. Berner, DDS, Cleveland, OH 44143*

**Learning Overview:** After attending this presentation, attendees will understand the advantages of implementing a safer dental sensor stabilization technique in conjunction with a sensor holder, to facilitate radiation exposure safety.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the application and use of “at hand” inanimate objects as stabilization devices during the capture of postmortem radiographic images, allowing operators to remove themselves from the field of radiation and thus reduce the risk of radiation exposure to response personnel.

**Background:** Dental radiograph sensor-positioning devices (X-ray sensor holders) are utilized in the office setting or in operating rooms during patient visits. These holders were developed for the routine care of a patient who is compliant and able to assist in the X-ray sensor stabilization process. In an adjunct application, these sensor holders have been employed in morgue settings to assist in the collection of postmortem radiographs.

Frequently during morgue operations, disaster response personnel attempt to use the same in-office devices on human remains to hold the radiograph sensor holder for a proper radiographic image to be obtained. Eighteen radiographs may be taken for a proper dental autopsy in the form of 4 bitewing and 14 periapical radiographs. If vertical or horizontal angulation corrections were made due to lack of anatomic landmarks or incomplete imaging, the operator tasked with sensor placement has often held the position during evaluation of the previous image. The central beam angles may have been corrected and another exposure accomplished. The various paths of the central beam would frequently put the computer terminal operator at risk of downrange exposure. If corrections in sensor placement were necessary, the sensor was adjusted by hand and held for the next exposure and the expected better image.

**Materials and Methods:** Controlled radiographic exposure and image capture of human remains were practiced by a small group of Disaster Mortuary Operational Response Team (DMORT) operators in a training Disaster Portable Morgue Unit (DPMU) setting with human remains. Technique repetition in a coroner’s office, and a later opportunity for training of response personnel with human remains, allowed for additional observations of this behavior change being easily accepted.

**Results:** The repeated effort to remove personnel from the downrange field of exposure was easily accomplished and became the basis for this study being applied to a larger group of responders. All personnel must stay cognizant of the exposure to one another, to stay flexible, and to move away from their station if the central beam would put them at risk of exposure. The use of devices allows the “wet” personnel to clear their hands from the field of radiation while the “clean and dry” data entry operators would step away, which reduces radiation exposure to themselves.

**Odontology, Identification, Mass Fatality**



## G36 A Cemetery Named the Mediterranean Sea: Best Practice in Human Identification

*Francesco Sessa, MS\**, Department of Forensic Pathology, University of Foggia, Foggia 71100, ITALY; *Stefania C. Bello, MD*, Ospedale Colonnello D'Avanzo, Foggia 71100, ITALY; *Giuseppe Bertozzi, MD*, Department of Forensic Pathology, Foggia 71121, ITALY; *Francesca Maglietta, MD*, Department of Forensic Pathology, Foggia 71122, ITALY; *Emilio Nuzzolese, PhD\**, Dental Team DVI Italia, Bari 70124, ITALY

**Learning Overview:** After attending this presentation, attendees will be able to evaluate the application of best practice when performing the identification of human remains recovered from the sea. There is a significant amount of scientific literature highlighting the importance of having a multidisciplinary approach for unidentified human remains by following Disaster Victim Identification (DVI) standards, yet they are not always applied.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the scientific findings of an identification case in which postmortem and antemortem data are compared to determine whether they belong to the same person. Since 2014, the human identification process has become an important procedure in Italy due to the migratory flows phenomenon and the large number of victims recovered from the Mediterranean Sea.

The European migrant crisis is a term given to a period beginning in 2014 when a rising number of people arrived in the European Union (EU), travelling across the Mediterranean Sea or overland through southeastern Europe. These people included asylum seekers, and also economic migrants.

Thousands of migrants die as a result of shipwrecks when trying to cross the Mediterranean Sea. This has caused the Mediterranean Sea to become a giant cemetery. In December 2014, a ferry boat, which routinely travels from Greece to Italy and vice versa, was set on fire. There were 443 passengers, 56 crew members, and at least 6 clandestine passengers on board. Due to sea conditions, it was not possible to rescue all passengers and, after the rescue, there were 19 missing passengers, 60 injured people, and 9 deaths. Four months later, a corpse in an advanced state of decomposition was recovered near the Tremiti Islands. It was not possible to determine the cause of death or the origin of the corpse. Was it a migrant, an accident, a homicide, or a suicide? Being of unknown origin, a general biological profile was achieved through a dental autopsy, fingerprints, and DNA collection.

One of the most challenging aspects of the human identification process is not the postmortem but the antemortem data collection. To begin gathering antemortem documents and data for identification, there is the need for “presumption of the identity,” which can be offered by the generic profile coming from the dental autopsy. The presumption of the identity was also obtained considering the circumstantial details of the report of the found body and analyzing the list of the missing people. This information was combined with the preliminary findings of the autopsy, such as the cause of death. A dental profile and genetic analysis (DNA and mitochondrial DNA (mtDNA) profiles) were also used to obtain significant data for the human identification process.

In general appearance, the recovered body appeared to be a male. The corpse wore only socks and underpants, and no other personal effects were found. Fingerprints were not practicable as the arms were not present due to sea fauna predation. The head was partially decomposed and the teeth and dental prosthetics were preserved. The histological findings guided the medicolegal analysis to determine that the person died by drowning. Dental X-ray analysis was also performed and was useful in determining the presence of an endodontic technique still used in Middle East European countries (i.e., Greece, Turkey), but no longer used by European clinicians.

Genetic analysis using Short Tandem Repeat (STR) allowed confirmation not only of the sex of the cadaver but also of the statistical distribution of the genetic profile in the population with Random Probability Match. Using mtDNA and an interactive map, it was also possible to obtain a profile for a database query that automatically directed to sampled populations: the haplotypes was R0a1a, the metapopulation was west Eurasian, the continent was Europe, specifically southern Europe, the country was Greece, and the province was Kozani. All data led to the ferry boat accident. A DNA comparison was then performed with the victims' DNA-available profiles. A DNA match was found for one of the missing person's relative's genetic profile; the corpse belonged to the one Grecian missing passenger.

This case report applied the multidisciplinary approach for best practice in human identification. Primary and secondary identifiers, following also DVI standards, could always be applied for the identification of migrant victims of the migratory flow through the Mediterranean Sea.

### Human Identification, Migrants, Dental Identification



## G37 Not Your Typical Forensic Odontology Cases

*John Berketa, PhD\*, University of Adelaide, Norwood, South Australia 5067, AUSTRALIA*

**Learning Overview:** After attending this presentation, attendees have information on various applications or skills of forensic odontology in relation to forensic sciences of which they have been unaware. These skills include evidence retrieval from the oral cavity, radiographic scanning of edentulous areas, and education of the public in the dental care of elderly citizens.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reminding attendees of how forensic odontology may be helpful beyond the traditional roles of restoration comparison in identification work, age estimation, and bitemark analysis. Three casework subject scenarios will be presented.

Regarding the first example, oral examinations conducted during identification work of victims was able to assist pathologists in determining the series of events that may have occurred in two possible homicide cases. Skin found between the teeth did not belong to the deceased in either case and was able to be linked to other suspects. Any material discovered within the oral cavity during a dental identification examination, especially biological material such as hair and skin, could be critical in investigations and need to be photographed *in situ*, recorded for chain of custody reasons, and, following approval from the attending pathologist or medical examiner, removed carefully for matching and/or further testing.

The second scenario suggests that edentulous alveolar arches should always be examined with full radiographic assessment. In a large disaster incident, full dental examination is routine. However, in a single victim case, a triage forensic officer or medical examiner may deem that odontologists may be of no benefit in the identification of an individual due to the edentulous state of the victim, and this process may be overlooked. Often, residual root tips or restorative material could be still present in an individual, which will be evident with dental radiological examination. This information may not be sufficient to confirm the identity of a person and could add further weight to the evidence already present. A casework illustrating this point will be presented.

The third scenario presents a tragic series of events in which a patient died due to denture asphyxiation. In this case, the elderly patient was severely medically compromised, and precautions, obvious to dentists, were not taken. Even though forensic odontology was not involved directly, it highlights the obligation the profession has to educate care workers and law makers regarding dental care and possible legislation.

Odontologists can contribute to the community in many ways, and it is important to keep an open mind for the unexpected ways in which they may assist.

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### Odontology, Unusual Cases, Investigations



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## G38 The Art of Working in Reverse to Resolve a Dental Identification

Mary K. Shields, DMD\*, Louisville, KY 40243; Mark L. Bernstein, DDS, University of Louisville School of Dentistry, Louisville, KY 40292

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**Learning Overview:** The goal of this presentation is to demonstrate how in dental identification, when efforts in which traditional methods have not produced sufficient antemortem evidence, reconstructive analysis of postmortem dental findings may provide a path to the recovery of additional data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing attendees to recent advances in dental restorative materials so they can understand the importance of knowing differences in their radiographic appearances. This can prove to be a valuable skill when performing a dental identification. Attendees will leave with a better understanding of how to identify modern dental materials radiographically. Modern materials, such as zirconia, porcelain, and lithium disilicate, have a variety of brand names and differ in the way they appear radiographically. Additionally, dental coding in patient charting can be vague regarding dental materials for prosthetics. This can pose a problem when comparing antemortem records and postmortem records.

This presentation will discuss roadblocks that complicated a seemingly straightforward dental identification and will also outline specific triumphs that led to a breakthrough in the identification effort by working backward.

An ideal dental identification of human remains can be simple when complete postmortem specimens having characteristic dental findings can be compared with promptly located and accessioned objective antemortem records mirroring those characteristics.

A family in the suburbs of a Louisville, KY, community notified the police after they stumbled across some bones in their backyard in a washed-out stream bed. A series of twists and turns ensued. Investigators found a backpack upstream with the name of a putative victim. A slow accumulation of antemortem evidence from a variety of sources increasingly supported only a possible identification. Finally, a reversed approach to acquiring antemortem data led to the resolution of the case.

Several considerations impacted the work of the forensic team: (1) recovery of remains (multiple searches); (2) media misreporting (multiple missing persons from the same area); (3) a search for antemortem records, including the family of the putative victim, hospital records, social media sleuthing with a search for smiling photographs, prison records, and working backward (i.e., reconstructive analysis); (4) knowledge of dental materials, specifically, fixed crown and bridge work; (5) sensitive subject matter; and (6) dental laboratory involvement (laboratory prescriptions as part of the dental records).

In cases in which dental identification efforts using only traditional methods have not produced sufficient antemortem evidence, reconstructive analysis of postmortem dental findings may provide a path to the recovery of additional data.

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### Dental Identification, Ceramic Crown, Radiographic Analysis



### G39 The Need for a Complete Dental Autopsy of Unidentified Edentulous Human Remains

Emilio Nuzzolese, PhD\*, Dental Team DVI Italia, Bari 70124, ITALY; Mario Torreggianti, DDS\*, Teramo, ITALY; Gina R. Quaglione, MD, UOC Anatomia Patologica, Teramo, ITALY

**Learning Overview:** After attending this presentation, attendees will understand the need for the application of best practices when conducting the identification of missing and unidentified human remains. The scientific literature in the field of human identification highlights the importance of a multidisciplinary approach when conducting a human identification process, including a dental autopsy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the results of an identification casework in which the dental autopsy and dental radiology were beneficial in the postmortem data collection, even in an edentulous individual, for the purpose of narrowing the search of reported missing persons in that geographical area and to establish identity.

In Abruzzo, one of the 20 regions of Italy with a total of more than 1.3 million inhabitants, there are, to date, 505 reported missing persons and 6 unidentified human remains as highlighted by the June 2018 official report of the Italian Ministry of the Interior.

In December 2017, a decomposed unidentified body was found near the river Tronto of Teramo, Italy. The corpse was found with no identifying document or specific personal belongings, except for a pack of cigarettes. The medical examiner diagnosed a gastric perforation as the cause of death, due to the intake of hydrochloric acid. The jar of muriatic acid found near the body led to a verdict of a suicide. After the autopsy, the Penal Court in Teramo appointed two forensic odontologists to complete the postmortem assessment and collect dental data for the purpose of identification. The cadaver was found wearing a complete set of upper and lower dentures. The dental autopsy and 43 periapical X-ray images determined the cadaver as a male between the ages of 55 and 65 years old, totally edentulous with upper and lower dentures, and an osteosynthesis with two plates and screws in the left corner of the mandible. The generic profile was circulated by the Carabinieri Police Agency and was also sent to Penelope Abruzzo, the regional association of missing persons, and to the editorial team of a national television program on missing persons, *Chi l'ha Visto?* (*Have You Seen This Person?*).

In March 2018, the sister of the missing person finally reported the disappearance of her brother, and a presumptive identification was performed through a visual recognition of the decomposed cadaver. The sister confirmed the presence of two dentures and revealed the name of the dentist and the place of the maxillofacial surgery for the treatment of the fractured mandible. The two forensic odontologists were able to establish the identification of the cadaver by comparing the dental and radiological data received from the dentist and the hospital. No DNA comparison was then required.

This case report confirms the need for always performing a complete dental autopsy, including of X-ray imaging, in all unidentified human remains cases, even in edentulous individuals in whom no teeth are clinically visible.

#### Identification, Edentulous, Dental Autopsy



### G40 A Review of Unconventional Points of Dental Identification at the Ontario Forensic Pathology Service: Thinking Outside the Viewbox

Yolanda Nerkowski, BA\*, Ontario Forensic Pathology Service, Toronto, ON M3M 0B1, CANADA; Robert E. Wood, DDS, PhD\*, Ontario Forensic Pathology Service, Toronto, ON M5G 2M9, CANADA; Taylor L. Gardner, BSc\*, Ontario Forensic Pathology Service, Toronto, ON M3M 0B1, CANADA

**Learning Overview:** The goal of this presentation is to demonstrate alternate or less commonly used points of dental identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining the use of unique dental identifiers in dental identification when more traditional points of dental identification are not available in the antemortem or postmortem data set.

The unique characteristics in the development, morphology, and pathology of dentition, coupled with their nearly indestructible quality, make teeth one of the most reliable means for establishing human identification. Additionally, well over half of North Americans are reported to have visited a dental professional in the past year, vastly improving the likelihood that antemortem dental records are available for comparison purposes should the need arise.<sup>1,2</sup> In addition, most people that have seen a dentist have some form of restorative intervention, be it dental restorations, root canal treatments, or extractions of permanent teeth.

The optimal scenario when performing a dental identification would be to have a postmortem data set containing multiple points of identification to compare against a complete antemortem data set, including radiographs. Although this is often the case, there are times when the antemortem or postmortem data set is lacking, either due to poor quality, lack of completeness, or the absence of antemortem records. In the postmortem data set, severe degradation of the body may require dental identification that relies on less traditional points of identification.

Several cases of unconventional points of identification will be discussed in which a positive dental identification was obtained or used to further support a circumstantial identification. One such identification was achieved using a photograph of the decedent's teeth taken antemortem demonstrating a rare feature of the crown in conjunction with other supportive points evident in the photograph, when antemortem dental records were not available. Age stratification was also undertaken to further aid in the establishment of this missing child's identity.

Dentures can prove to be of considerable value in identification as they may contribute to the creation of a biological profile for unidentified human remains. Wear patterns, materials used in the manufacturing, and the overall quality of the dental prosthesis can yield important clues to an unidentified person's habits, age, postmortem interval, or geographical region of origin. Dentures can also be fitted on casts in cases of presumptive identification when these items can be obtained from an individual's dentist. Unfortunately, dental casts are not always available, and a more novel approach must be taken to reveal the utility of a denture in establishing identification. One such case involved an edentulous individual whose identification was made possible by corroboration between the dentist's notes regarding a denture adjustment and an examination of the denture and shade-matching of the appliance.

Finally, several cases with irregular anatomical features or unusual pathoses will be presented in which either peri-mortem destruction or loss of the dentition, or edentulism, has made identification using more commonly used identifiers difficult. These cases include an individual with a unique unilateral nasopalatine duct cyst, a maxillary fracture, and comparison of the left maxillary sinus seen in postmortem Computed Tomography (CT) imaging to antemortem radiographs.

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2. Centers for Disease Control and Prevention, National Center for Health Statistics, *Oral and Dental Health* (2016). Retrieved from <https://www.cdc.gov/nchs/fastats/dental.htm>.

#### Dental, Identification, Unconventional

## G41 The Lethal Migrant Sea Route to Italy and the Nigerian Disaster Victim Identification (DVI) Operation

Antonello Crisci, MD, University of Salerno, Salerno 84084, ITALY; Emilio Nuzzolese, PhD\*, Dental Team DVI Italia, Bari 70124, ITALY; Joe Adserias-Garriga, DDS, PhD\*, Texas State University, Forensic Anthropology Department, San Marcos, TX; Margherita Neri, MD, PhD, University of Ferrara, Ferrara 44100, ITALY

**Learning Overview:** After attending this presentation, attendees will have a deeper understanding of how victims of migratory flow should be managed, how important teamwork is, and the application of the International Criminal Police Organization (INTERPOL) standards.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the complete rescue, recovery, DVI, and burial operations of 26 bodies drowned in the Mediterranean Sea while migrating to Italy.

On the November 5, 2017, a migrant boat with 375 Nigerian migrants was rescued in the Mediterranean Sea by the Spanish military ship *Cantabria*. Twenty-six Nigerian women and girls were found dead from drowning. The boat with migrants departed from Libya but began sinking near the Italian coast because it was overloaded. Dead bodies were found floating in the water, while survivors clung to partly capsized rubber dinghies. At least 100 bodies are believed to be still missing in the sea.

A DVI operation was established in Salerno, where the *Cantabria* was due to dock. The forensic pathologist appointed by the Penal Court in Salerno promptly set up a team of other forensic pathologists, odontologists, and anthropologists. Personal belongings and all identifying traits (tattoos, scars, and anatomical variants) were recorded during the first preliminary external examination of the dead bodies. Autopsies were conducted at the local cemetery with the goal of diagnosing the cause of death and retrieving all possible data of potential criminal acts, such as torture or violence. Two girls were found to be pregnant, and the cause of death was drowning for the whole group.

Dental autopsies were performed according to INTERPOL standards, with the principal goal of assessing the age of the victims. Age estimation was assessed using Mincer et al., AlQahtani et al., and Carmeriere et al., through the periapical radiographs of canines and third molars, using a portable X-ray hand-held device.<sup>1-4</sup> The age assessment revealed a demography of five minors and 21 adults. Dental examinations and autopsies were completed in three days for the purpose of speeding up the entire operation. Unusual findings will be presented, including a SIM card, ethnical identifying tattoos and scars, and four bitemark-patterned lesions on two of the victims. State funerals were held on November 17, 2017, with a Catholic and Muslim ceremony in the presence of the surviving Nigerian migrants, authorities, and citizens of several nationalities.

Since 1993, the number of migrants whose deaths have been reported is 34,361.<sup>5</sup> In addition, there are migrants whose deaths go undocumented, because they are lost at sea or die in the backs of trucks. These deaths can be described as a humanitarian mass disaster, rather than simply a natural mass disaster. The highest number of deaths occurred in the Mediterranean Sea, considered the most lethal sea route to Italy. From the forensic perspective, humanitarian forensic odontology can offer a strong contribution to migratory flows and illegal migration, reinforcing public health assessment, border control, and identification of the living and the deceased.

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### Migrants, Humanitarian Forensic Odontology, Dental Age Estimation





### H1 Stress Evaluation in Indonesian Civil Aviators

*Meta Y. Candra, MSc\**, Faculty of Dentistry Universitas Indonesia, Jakarta 10430, INDONESIA; *Nurtami Soedarsono, PhD*, Faculty of Dentistry Universitas Indonesia, Jakarta, DKI Jakarta 10430, INDONESIA; *Dewi F. Suniarti, PhD*, Faculty of Dentistry Universitas Indonesia, Jakarta 10430, INDONESIA; *Febriana Setiawati, PhD*, Faculty of Dentistry, Jakarta 10430, INDONESIA

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**Learning Overview:** Flight safety needs to be improved. After attending this presentation, attendees will understand how the work of a civil aviator is a formidable challenge and stress risk. A biological screening to evaluate civil aviators' stresses to minimize the human risk factor will be reported in this presentation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by encouraging preventive measures to keep civil aviators from being incapacitated and methods to investigate a human risk-factor involvement in an aviation incident. It is important to take notice at this issue and develop early physical and physiological assessments on civil aviators.

Due to the high level of civil aviation incidents in Indonesia from 2010 to 2017, it is necessary to evaluate human factor as one of the main culprits. A civil aviator's stress condition can arise from aviation environments, such as altitude, noise, communication, and vibration. A civil aviator, who operates a short-haul flight in Indonesia (an archipelagic country), will routinely have many flying hours that may turn into a stressor. It was predicted that an aviator's stress is caused by fatigue. The International Civil Aviation Organization (ICAO) uses the Fatigue Severity Scale (FSS) method to measure the level of clinical fatigue in a person by using nine questionnaires with a score of 1 to 7 for the level of fatigue.

This study measured the level of clinical fatigue using the FSS on aviators who do not fly sectors and have a total flight time of less than 6,624 hours, and those who are on a sector flight and have a total flight time of more than 6,624 hours. There are five proteins secreted during stress that can be used as stress biomarkers: cortisol, lysozyme, immunoglobulin A (IgA), chromogranin, and alpha amylase. This study evaluated the stress condition of Indonesian civil aviators using salivary alpha amylase and blood cortisol as biomarkers on both groups.

From this study, it was concluded that stress on civilian aviators can occur because of the high level of fatigue caused by the total number of flying hours that exceed the specified limits. In addition, more than two sector flights within 24 hours also caused aviator fatigue. This fact has been demonstrated by the increased salivary alpha amylase and blood cortisol levels and FSS scores in the aviation group with total flight hours exceeding 6,624 hours and who performed sector flights.

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### Fatigue, Stress, Civil Aviator

## H2 The Application of Decomposition Scoring Methods to Predict Postmortem Submersion Interval (PMSI) in Human Bodies Recovered From Aquatic Environments: A Comparison Between Freshwater and Salt Water in Italian Areas

Chiara Palazzo, MD\*, Bologna, ITALY; Paolo Fais, PhD, Department of Medical and Surgical Sciences, Unit, Bologna, ITALY; Guido Pelletti, MD, Department of Medical and Surgical Sciences, Bologna, ITALY; Alberto Amadasi, MD, Università di Bologna, Bologna 40126, ITALY; Federica Fersini, Bologna, ITALY; Filippo Pirani, MD, Section of Legal Medicine, Polytechnic University, Ancona, ITALY; Rosa Maria Gaudio, University of Ferrara, Ferrara 44121, ITALY; Adriano Tagliabracci, MD, Section of Legal Medicine, Polytechnic University, Ancona, ITALY; Susi Pelotti, MD, University of Bologna, Bologna 40100, ITALY

**Learning Overview:** After attending this presentation, attendees will understand the applicability of aquatic decomposition scoring methods to predict PMSI in human bodies recovered from Italian rivers and the Mediterranean Sea near Italian coasts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by aiding in the estimation of PMSI in submerged corpses in temperate climatic areas.

Determining PMSI may be problematic, since an aquatic environment can affect the rate of postmortem decay. In several studies, performed in northern Europe, PMSI has been estimated through scores that evaluate visual postmortem modification on the head and neck, trunk, and limbs. Based on the scoring system developed by Megyesi et al., Heaton et al., and van Daalen et al. recently developed two new scores to assess aquatic decomposition.<sup>1-3</sup> Basing on the transformative processes observed on head and neck, trunk, and limbs, a Total Aquatic Decomposition Score (TADS) is calculated and related to PMSI. Moreover, since aquatic decomposition is time and temperature dependent, TADS has also been related to Accumulated Degree Day (ADD), which is the sum of average daily temperatures of the body of water, along with the submersion interval.<sup>2-4</sup>

Different environmental conditions and ecosystems influence the rate of decomposition and the relationship between TADS, PMSI, and ADD. Therefore, there is a growing need for systematic studies on human decomposition in aquatic environments.

The goal of this study is to evaluate whether the scores developed by Heaton et al. and van Daalen et al. are suitable in estimating the PMSI in bodies recovered from aquatic environments in a temperate area, namely Italian rivers and the Mediterranean Sea, near the Italian coasts.<sup>2,3</sup>

Both scores were tested on 285 bodies recovered from freshwater and 45 bodies recovered from the sea, in which PMSI was known. The degree of decomposition within the study samples was heterogeneous, varying from no visible changes to almost complete skeletonization. Postmortem decomposition was assessed using photos taken during death scene investigations. Aquatic temperature to calculate ADD was assessed by referring to official weather databases. The scores obtained through the application of the scales were compared with the real PMSI and the related real ADD values. Regression analysis was conducted to evaluate the accuracy of TADS in predicting the PMSI, according to the following logical frame: (1) TADS according to Heaton et al.'s score vs PMSI; (2) TADS according to Heaton et al.'s score vs ADD; (3) TADS according to van Daalen et al.'s score vs PMSI; and (4) TADS according to van Daalen et al.'s score vs ADD.

The correlation between the TADS and PMSI was calculated and a high correlation was observed, especially for van Daalen et al.'s score. The different regression coefficients obtained in relation to the freshwater cases and the salt water cases confirm the influence of the different aquatic environments on the rate of postmortem decay.

According to this study, both scoring methods can be applied to bodies recovered from fresh and salt aquatic environments within the Mediterranean area, taking into consideration the climatic features with periodical temperature variation, to achieve the best accuracy in predicting PMSI.

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### PMSI, Decomposition, Score



## H3 Unusual Asphyxial Deaths: Sand, Soil, and Stool

*Diana C. Nointin, MD\**, Maryland Office of the Chief Medical Examiner, Baltimore, PA 21223; *Pamela A. Ferreira, MD*, Columbia, MD 21044; *Carol Allan, MD*, Office of the Chief Medical Examiner, Baltimore, MD 21223; *Zabiullah Ali, MD*, Office of the Chief Medical Examiner, Baltimore, MD 21223; *David R. Fowler, MD*, Office of the Chief Medical Examiner, Baltimore, MD 21223

**Learning Overview:** The goal of this presentation is to report a series of unusual asphyxial deaths due to the inhalation of foreign material.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the characteristics of asphyxial death caused by the inhalation of foreign material and the need to standardize the classification of asphyxial deaths.

A 30-year-old female was found buried in sand at the beach. The autopsy showed sand in the eyes, ears, nose, sphenoid sinuses, oral cavity, and upper and lower airways. A Computed Tomography (CT) scan showed high-density material in the bronchial tree.

A 26-year-old male who was working at a construction site was witnessed to be buried in soil when the 8-foot hole he was digging collapsed on him. The autopsy showed collections of sand and dirt in the nose, oral cavity, upper and lower airways, esophagus, and stomach.

A 20-year-old male was working in an excavation site repairing a storm drain when the site started to collapse. The autopsy showed collections of mud and dirt in the upper airway, abrasions and contusions of the face, neck, and left arm, and lacerations on the right posterior scalp.

A 48-year-old male (A), an 18-year-old male (B), and a 14-year-old male (C) were found in a manure pit at a dairy farm. All three were working at the farm spreading manure on the fields before they were reported missing. The autopsies showed liquid and semisolid manure in the upper and lower airways and sphenoid sinuses on all three cases. Testing for potential methane gas presence in the lungs was unable to be performed due to impaction of semisolid stool in the airways.

Asphyxia is a broad term used to describe the disruption of oxygen uptake or utilization in cells. Asphyxia can be further subcategorized into suffocation, mechanical asphyxia, strangulation, and drowning.<sup>1</sup> Additionally, the term suffocation is non-specific and encompasses a variety of mechanisms that include smothering, choking, and confined spaces/entrapment/compromised atmosphere, each with its own definition and criteria. Over the past ten years, there were 2,055 deaths attributed to asphyxia at the Maryland Office of the Chief Medical Examiner, including those due to drowning (904 deaths), strangulation/hanging (406 deaths), suffocation (218 deaths), and mechanical asphyxia (143 deaths). The remaining 384 deaths were non-specified, which included the 6 reported cases above. Most of these reported cases showed overlap between categories, including obstruction of the upper airways (smothering) and lower airways (choking) by foreign material, and all likely have impaired respiration due to pressure from being buried or submerged (mechanical asphyxia). In four out of six cases, permeation of material into the sphenoid sinuses was present, which is usually seen in cases of drowning. These cases highlight the apparent complexity of asphyxial deaths and the possible variation of categories in which they may be classified. Although deaths due to inhalation of foreign material are uncommon, they show similar findings at autopsy and likely share the same mechanisms of death. Recognition of these presentations and further exploration of similar cases can aid in structuring a scheme to provide better standardization under the current classification system.

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### Asphyxia, Suffocation, Drowning



### H4 Strangulation to Stroke: A Fatal Case of Delayed Traumatic Internal Carotid Artery Dissection

*Amber R. Wang, MD\*, Maricopa County Medical Examiner's Office, Phoenix, AZ; Alan C. Wang, MD, Jackson Memorial Hospital, Department of Neurology, Miami, FL 33136; Benjamin Mathis, MD, Miami-Dade County Medical Examiner Department, Miami, FL 33136*

**Learning Overview:** After attending this presentation, attendees will be aware that carotid artery dissection is an uncommon, delayed presentation of strangulation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of an uncommon mechanism of death occurring as a result of neck manipulation and/or trauma. This case stresses the importance of obtaining a thorough history for previous trauma in cases of carotid artery dissection and stroke to properly certify these deaths.

Traumatic carotid artery dissections are uncommon and can be fatal injuries with a delayed onset. The time between neck trauma and neurological signs can be hours to years following the initial injury.

A 32-year-old female presented to the emergency room after being sexually and physically assaulted and strangled. Upon physical examination, she had multiple contusions and lacerations, subconjunctival hemorrhage bilaterally, and a faint ligature mark predominantly around the right side of her neck. A Computed Tomography (CT) of the brain was normal. She was awake and alert. The woman was interviewed by police. Approximately four hours later, she developed an acute left hemiparesis and aphasia. A repeat CT scan of the head showed an infarct in the right middle cerebral artery territory. A Computed Tomography Angiography (CTA) demonstrated a complex dissection of the right internal carotid artery with thrombus extending into the middle cerebral artery. Despite multiple attempts at mechanical thrombectomy and neurosurgical intervention, she was declared brain dead and expired four days later. An autopsy confirmed the cause of death as cerebral infarction due to right internal carotid artery dissection due to ligature strangulation, and the manner was certified as homicide.

Carotid artery dissection is rare but is the most common cause of stroke in people under 45 years of age. It can occur spontaneously or secondary to trauma. Spontaneous dissections are often associated with connective tissue disorders, such as fibromuscular dysplasia. The majority of traumatic carotid artery dissections are due to high-speed motor vehicle accidents, sports, falls, and violent activity. The mechanism by which traumatic carotid artery dissection occurs is hypothesized to be due to stretching of the artery during neck hyperextension, compression against the spinal column during contralateral flexion of the neck, and direct blunt trauma from a blow or other external force. When the dissection occurs, a thrombus may form nearby and result in distal embolism or occlusion.

Cerebral infarction following dissection can be immediate or significantly delayed with respect to the decedent's initial presentation. The interval from neck trauma to onset of neurological symptoms is generally considered to be within 24 hours. However, multiple reports in the literature describe intervals of months and, rarely, even years. Despite medical treatment, including anticoagulation therapy, resulting in a high percentage of spontaneous resolution, a subset of dissections may progress to a chronic stenosis, complete occlusion, or a pseudoaneurysm with risk of rupture. Thus, the association of arterial pathology with the inciting event may be lost due to the delay in presentation.

This case highlights the danger of delayed stroke as a consequence of strangulation, a phenomenon rarely described in the forensic literature. Acquisition of a complete history regarding previous trauma, especially neck pressure or manipulation, is necessary information required for proper certification of deaths related to carotid artery dissection and/or ischemic stroke.

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#### Strangulation, Carotid Artery Dissection, Stroke



## H5 A Fatal Case of Candy Aspiration in a Child: Was It a Choking Death?

Giuseppe Davide Albano, MD\*, Foggia 71121, ITALY; Pasquale Malandrino, MD, University of Catania, Catania 95123, ITALY; Massimiliano Esposito, MD, University of Catania, Catania 95123, ITALY; Aldo Liberto, MD, University of Catania, Catania 95123, ITALY; Dario Condorelli, University of Catania, Catania 95100, ITALY; Monica Salerno, MD, PhD, Department of Forensic Pathology, Foggia 71121, ITALY; Giulio Di Mizio, MD, PhD, Magna Graecia University, Catanzaro 88100, ITALY

**Learning Overview:** The goal of this presentation is to describe characteristics, circumstances, radiological, toxicological, and histopathological findings of a singular case of a Foreign Body Aspiration (FBA) death in a child.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting the necessity for a complete methodological and multidisciplinary forensic approach by means of autopsy and histopathological examinations to diagnose an FBA-related death.

FBA is still a significant concern in the pediatric population, accounting for thousands of emergency room visits and more than 150 deaths each year in the United States alone.<sup>1</sup> Mortality rates can be relatively high, with some studies reporting a rate of up to 3.4% among patients admitted for FBA.<sup>2</sup> Depending on the location and the degree of airway obstruction, patients can present with signs and symptoms ranging from a non-specific cough to shortness of breath and stridor. In case of distal localization in the tracheobronchial tree, the most common symptoms are unilateral wheeze and decreased breath sounds.<sup>3</sup> Even a small reduction in the size of airway can cause a significant increase in airway resistance; therefore, the consequences of foreign body inhalation could be dramatic.<sup>4</sup> This report describes characteristics, circumstances, radiological, toxicological, and histopathological findings of a singular case of an FBA-related death in a child.

This case regards a 7-year-old child who died at home after a licorice candy ingestion. After the candy ingestion, he started coughing and wheezing and immediately turned cyanotic. His mother attempted resuscitative procedures that were unsuccessful and called the emergency services. At arrival, emergency services attempted tracheostomy without success and decided to transport the child to the closest hospital. During transportation, inside the ambulance, death was pronounced. Recent medical history was positive for an airway infection: cough and mucus were present and he had been treated with antibiotics for five days.

A Computed Tomography (CT) total body scan performed prior to autopsy excluded any traumatic lesion as well as the presence of foreign bodies in the upper airways and the main bronchi.

A complete autopsy was performed two days after death. Cervical and thoracic organs were dissected with Gohn's method (*en bloc*). Inside the trachea and main bronchi a brownish dense material and white foam were observed; in the right bronchus such material was denser. The trachea and main bronchi walls had no lacerations. Macroscopic examination of the brain and abdominal organs was unremarkable. After fixation, the cervical and thoracic organs *en bloc* was examined. Inside a secondary right bronchus, a black, foreign body of hard consistency, which measured 0.5cmx0.4cm, was observed. Histologic examination of all organs was performed using Hematoxylin-Eosin (H&E). An immunohistochemical staining method of lung samples was performed with CD45 and mast-cells antibodies. H&E-stained lung samples revealed acute emphysema, endoalveolar hemorrhagic oedema, as well as thickening of alveolar septa. H&E-stained bronchi and trachea samples revealed thickening of the wall and signs of chronic lymphocytic inflammation. H&E samples of all organs revealed the presence of lymphocytic inflammation. Immunohistochemical staining of lung samples showed a strong positive reaction to CD45 and mast-cells antibodies with signs of degranulation. Routine toxicological analysis was performed and was unremarkable.

In conclusion, the circumstantial data, both macroscopic and microscopic upper airways and lung findings as well as immunohistochemical study, contributed to exclude a choking death. Cause of death was attributed to an acute respiratory failure due to a bronchospastic reaction after a foreign body aspiration in a child with chronic systemic lymphocytic inflammation.

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### Foreign Body Aspiration, Asphyxiation, Choking



### H6 Lichtenberg Figures in Electrocution Deaths by Evaporative Cooler

*Nilesh K. Tumram, MD\*, Department of Forensic Medicine and Toxicology, Nagpur, Maharashtra 440013, INDIA*

**Learning Overview:** After attending this presentation, attendees will understand that Lichtenberg figures, until now synonymous with lightning strikes, can also occur in electrocution caused by electrical instruments, such as evaporative coolers. This presentation will discuss the possible mechanism of causation of Lichtenberg figures in conditions other than lightning strikes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the occurrence of Lichtenberg figures after electrocution by an evaporative cooler.

In a bizarre accident, three persons of the same family were found electrocuted by the evaporative cooler at their home. The incident happened when a young lady in her 30s, the daughter of an elderly couple, was replacing water in an evaporative cooler. Incidentally, the evaporative cooler was kept running while she was filling it with water. As the water flowed into the evaporative cooler, some spilled on the floor. Though initially everything went smoothly, when the water was spilled on the ground, the lady suddenly screamed for help. Hearing her scream, her mother and father rushed near the evaporative water cooler where their daughter was found lying on the ground and screaming for help. Without any hesitation, the couple went to pull her up, but they also stepped in the pool of water on the floor and started to scream. In the meantime, the neighbors sensed trouble. When the neighbors reached them, they found all three people lying in the pool of water, with the evaporative cooler fallen on one side. The neighbors carefully switched off the mains of the electric source of building, then tried to wake them. However, nobody dared go over the wet floor in fear of themselves being electrocuted too. Someone called the police, who came after some time and found the victims already dead.

The bodies were subjected to a medicolegal autopsy. It was observed that the young lady received superficial burns over her entire back, with blackening and peeling of the skin. There were also abrasions over her chin, right cheek, the right side of her mandible, and the left side of her forehead. There was flattening of the lateral aspect of the left heel, a pattern of reddening tree branching/Lichtenberg figures over the left sole, and the right sole was completely pale. There was cyanosis of the fingernail beds and the internal organs also showed multiple petechial hemorrhages in the lungs and heart.

Interestingly, barring mechanical injuries, there were similar Lichtenberg figure patterns over the soles of the two other victims. Such types of Lichtenberg patterns are common in deaths or accidents by lightning strikes. However, a Lichtenberg figures pattern in electrocution deaths other than lightning is not known per this study's research.

#### **Lichtenberg Figures, Evaporative Cooler, Electrocution**



### H7 Modeling of Inflicted Head Injury by Shaking Trauma in Children—What Can We Learn? Part 1: A Systematic Review of Animal Models

*Marloes E.M. Vester, MD\**, Netherlands Forensic Institute, The Hague, ZH 2497GB, NETHERLANDS; *Rob A.C. Bilo, MD*, Netherlands Forensic Institute, The Hague 2490 AA, NETHERLANDS; *Arjo J. Loeve, PhD*, Delft University of Technology, Delft, Zuid-Holland 2628 CD, NETHERLANDS; *Rick R. Van Rijn, PhD*, Amsterdam UMC, Amsterdam 3544MT, NETHERLANDS; *Jan Peter van Zandwijk, PhD*, Netherlands Forensic Institute, The Hague, Zuid Holland 2497 GB, NETHERLANDS

**Learning Overview:** After attending this presentation, attendees will understand the different forces and their respective injuries in animal studies with lambs and piglets mimicking inflicted head injury without any direct external impact.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a systematic review to address the pathophysiology in order to discuss the forces needed to cause injury in infant animals. These values are important in the interpretation of injury in alleged human abusive head trauma cases.

Injury in infants caused by repetitive acceleration-deceleration trauma (e.g., caused by shaking) in the absence of a direct impact trauma to the outside of the head, other than chin-chest and head-back collisions, is a process better defined as Inflicted Head Injury caused by Shaking Trauma (IHI-ST). IHI-ST, also commonly referred to as Abusive Head Trauma (AHT), can cause brain injury. Yet, the exact pathophysiologic mechanism and its associated thresholds remain unclear. In this systematic review, an overview of animal model studies for shaking injury and their findings on tissue damage will be provided.

A systematic review was performed in MEDLINE® and Scopus® for articles on the simulation of inflicted head injury in animals, up to January 1, 2017. After collection, the studies were independently screened by two researchers for title, abstract, and finally full text, and on methodological quality.

After de-duplicating of the search results, 1,977 articles remained, resulting in 23 articles eligible for full text screening. A total of 12 articles were included after full text screening. Three articles were based on a single study population of 13 lambs, all of one research group. The other 9 articles were separate studies performed in piglets, all by a single second research group.

The 5-to-10-day-old, anesthetized lambs were vigorously shaken by adult humans, resembling human infant shaking, and euthanized after six hours. Overall, the shaken lambs showed injuries of the brain, spinal cord, and eyes. The control animals did not show any relevant abnormalities. Injury was more common and more extensive in the lower weight, younger lambs, who all died prematurely.

In five studies, 3-to-5-day-old piglets received a rapid inertial, non-impact head acceleration in the transverse (axial) plane, while three other articles used accelerations in different planes (transverse, sagittal, or coronal). Lastly, one article reported solely on transverse accelerations as well, yet in 4-week-old piglets. In piglets, it was found that rotation direction was of influence on the neurological and histology results. Tissue strain might be of influence on the injuries found in piglets and lambs. However, the anatomical differences between animals and humans, along with the inconsistent choice of (mostly non-cyclic) rotation directions in the various studies make an adequate comparison between studies and between animals and humans very difficult.

The lamb articles give some information on tissue damage after inflicted head injury. The piglet studies only provide information on consequences of a single plane rotational movement. Generally, with increasing age and weight, there was a decrease of axonal injury and death. Future studies should focus on every single step in the process of a free head movement in all directions, resembling human IHI-SC. In part II of this systematic review, biomechanical models will be evaluated.

#### Closed Head Injuries, Child Abuse, Animal Models



### H8 Modeling of Inflicted Head Injury by Shaking in Children—What Can We Learn? Part 2: Mathematical and Physical Models

*Jan Peter van Zandwijk, PhD\*, Netherlands Forensic Institute, The Hague, Zuid Holland 2497 GB, NETHERLANDS; Arjo J. Loeve, PhD, Delft University of Technology, Delft, Zuid-Holland 2628 CD, NETHERLANDS; Marloes E.M. Vester, MD, Netherlands Forensic Institute, The Hague, ZH 2497GB, NETHERLANDS; Rob A.C. Bilo, MD, Netherlands Forensic Institute, The Hague 2490 AA, NETHERLANDS; Rick R. Van Rijn, PhD, Amsterdam UMC, Amsterdam 3544MT, NETHERLANDS*

**Learning Overview:** After attending this presentation, attendees will: (1) be familiar with the different types of mathematical and physical models that have been used to investigate inflicted head injury by shaking in children, (2) learn about the strengths and limitations of these models and their underlying injury thresholds, and (3) learn how to put into perspective the various claims in the field about inflicted head injury by shaking in children.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing medical and legal practitioners who are not biomechanically trained to better put the claims made in the literature into perspective. This is crucial for proper interpretation of injuries in alleged abusive head trauma cases.

Various types of complex biomechanical models have been published in the literature to better understand processes related to Inflicted Head Injury by Shaking in Children (IHI-CS). The wide variety of model types and modelling approaches makes it hard to compare these models to each other and value their—sometimes contradictory—conclusions with respect to the likelihood of pediatric head injury occurring due to shaking. A systematic review was conducted to categorize the available modelling studies and to enable better comparison of these studies.

MEDLINE® and Scopus® were used to find studies applying physical (such as dolls) and mathematical (computer simulation) biomechanical models for shaken induced head injury in children up to January 1, 2017. After de-duplication, the found articles were independently screened by two researchers using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) approach. Data extracted from the papers is represented in a “7-Steps Description” of IHI-CS, addressing the sequence of mechanical events that take place in a child subjected to shaking.

The initial database search yielded 1,977 papers, of which 11 papers on physical models and 23 papers on mathematical models were included after the selection process. In both categories, some models focus on describing gross head kinematics during shaking events, while others address the behavior of internal head and eye structures in various levels of detail.

In the 7-Steps Description, an IHI event is initiated with a shaking motion exerted onto the infant’s torso: the “torso dynamics,” which is the input at Step 1. That motion is transferred by the neck to the head in Step 2 “torso-skull transfer,” which results in the “skull dynamics” at Step 3. In turn, these skull dynamics determine in Step 4 “skull-internal transfer,” how everything inside the skull moves and deforms, resulting in the “internal dynamics” at Step 5. Internal dynamics include loading and deformation of the anatomical elements inside the skull, such as pressure acting on the eyes or strains acting on the bridging veins. The dynamics of internal anatomical elements may lead to damage of these elements if their threshold for material damage is exceeded in Step 6 “injury thresholds,” which then gives rise to “injury” in Step 7.

In virtually all mechanical and mathematical models analyzed, injury thresholds were derived from scaled non-infant data. Studies focusing on head kinematics often used injury thresholds derived from impact studies. Physical modelling studies as well as some mathematical models focusing on head kinematics often conclude that shaking alone cannot produce injury. However, mechanical models containing detailed head anatomy, as well as mathematical models containing a detailed description of structures inside the infant head and eye suggest that shaking events can indeed produce injuries, such as bridging vein rupture and retinal hemorrhaging. Besides this, several of the studies analyzed hinted at the possibility that the injury mechanism during shaking is fundamentally different from that during impact. Mechanical effects in which shaking likely differs from impact include build-up of pressure within the head over multiple shaking cycles, resonance effects, and a slower, but potentially higher, transfer of deformation energy.

Future research focusing on determining injury thresholds for infant biological material is urgently called for. This holds both for macroscopic anatomical data, such as stiffness of the infant neck, as well as for microscopic data, such as the failure stretches of infant bridging veins. Also, possible other injury mechanisms during shaking should be further investigated, because there seem to be clear indications that the currently often-made comparisons of shaking events with impact injury thresholds may not be valid.

#### Closed Head Injuries, Child Abuse, Rigid Body and Finite Element Model





### H9 Chillin' in Hotlanta: A Five-Year Retrospective Study of Hypothermia Deaths in Fulton County, Georgia

Maxwell O. Rollins, MD\*, Fulton County Medical Examiner, Atlanta, GA 30312; Jan M. Gorniak, DO, Fulton County Medical Examiner, Atlanta, GA 30312; Jason Singh, MD, Emory Pathology, Atlanta, GA 30322

**Learning Overview:** The goals of this presentation are to: (1) examine risk factors for hypothermia, and (2) assess anatomic findings of hypothermia.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying risk factors for hypothermia that may help the population in preventing hypothermia.

Hypothermia-related deaths are prevalent during the winter in states that have cold climates; however, hypothermia-related deaths also occur in states with milder climates. Close to 800 hypothermia deaths occur each year in the United States. According to the Center for Disease Control and Prevention (CDC), hypothermia is clinically defined as lowering of the core body temperature to less than or equal to 95°F (less than or equal to 35°C). Exposure to cold is responsible for approximately twice the number of deaths annually as is exposure to heat in the United States.

All records of deaths occurring in Fulton County, GA, from 2013 to 2017 in which hypothermia was listed as the cause of death were reviewed. Review of the 47 cases showed that all hypothermia deaths were ruled accidents. There were 41 (87%) male decedents and 6 (13%) female decedents. At least 28 (60%) of the decedents were homeless. Thirty-eight cases (81%) occurred in the outside environment and 9 cases (19%) occurred inside a building. Of the cases that occurred inside ( $n=9$ ), four (44%) occurred in vacant buildings and 5 (55%) occurred in the decedent's own residence. Ethanol was associated with 15 (32%) cases (with ethanol alone or in combination with other drugs), with an ethanol (vitreous or blood) concentration ranging from 74mg/dL to 488mg/dL (.074%–.488%). Of the autopsied cases ( $n=43$ ), grossly, gastric mucosal petechial hemorrhages/hemorrhagic ulcers (Wischnewski spots) were identified in 20 cases (47%).

The year 2014 had the highest number of cases ( $n=18$ , 38%), although surprisingly, no specific correlation is seen with the winter storm that crippled Atlanta for nearly a week beginning January 28, 2014, known colloquially as "Snowmageddon 2014" (in the seven days from 1/28/14 to 2/4/14, there were four cases of hypothermia). It is not known why 2014 had almost 40% of the Fulton county cases over a 5-year period, although per CDC data, nationally in 2014, there were more hypothermia deaths than in the prior 13 years. Understanding risk factors of hypothermia in Fulton County and on a national level is essential for improving prevention efforts.

#### Hypothermia, Weather-Related Death, Drugs of Abuse



### H10 “Hot Deaths”: A Review of Foggia Hyperthermia Cases

*Stefania De Simone, MD\**, Department of Forensic Pathology, Foggia 71122, ITALY; *Francesca Maglietta, MD*, Department of Forensic Pathology, Foggia 71122, ITALY; *Lorenzo Spagnolo, MD*, Department of Forensic Pathology, Foggia 71122, ITALY; *Santina Cantatore, Foggia 71100, ITALY*; *Pietrantonio Ricci, MD, PhD*, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY; *Monica Salerno, MD, PhD*, Department of Forensic Pathology, Foggia 71121, ITALY; *Francesco Sessa, MS*, Department of Forensic Pathology, Foggia 71100, ITALY; *Giuseppe Bertozzi, MD\**, Department of Forensic Pathology, Foggia 71121, ITALY

**Learning Overview:** The goal of this presentation is to show that in deaths related to hyperthermia, even if caused by different situations, it is possible to reach a diagnosis through the study of some microscopic findings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that similar microscopic evidence can be found in heat-related deaths, suggesting the use of these postmortem analyses in similar cases.

Hyperthermia is a condition characterized by an uncontrolled increase in body temperature, which the human body can not dissipate. Exposure to excessive heat usually determines an activation of the hypothalamic thermoregulatory centers, which are activated to favor the dispersion of heat through vasodilatation and sweating. If this does not happen, there is a disruption of the thermoregulation systems, and, therefore, there is rapid multi-organ failure, which leads to death.

This study presents three cases of fatal hyperthermia, analyzed by the Department of Forensic Pathology of Foggia. The first case concerns a 26-year-old Romanian girl, treated for a depressive disorder, who was found immersed in hot water in a bathtub. The toxicological analyses performed on the girl's blood revealed the presence of high concentrations of alcohol. The intake of high doses of alcohol leads to a state of deep lethargy; therefore, the girl, not feeling the excessive heat of the water, in a state of acute intoxication, could not get out of the bathtub. During the crime scene investigation, conducted six hours after the discovery of the corpse, the water temperature was 30°C, while the cadaveric temperature was 43°C.

The second case is that of a 51-year-old man who worked aboard a fishing boat. During a very hot day, around 2:00 p.m., he began to be confused, with garbled speech, until losing consciousness shortly thereafter. Death was attributed to a heat stroke.

The third case is related to a 47-year-old man, a prisoner and ex-drug addict in methadone and neuroleptics therapy, who was found lying on the bed with a high fever while wearing nine wool sweaters. Subsequently, he was transported to the hospital in a comatose state, with a body temperature of 42.8 °C; he died a few hours later.

Presented are three different causes of death related to hyperthermia (immersion of the body in hot water, heat stroke, and intake of neuroleptics), but these all have in common some histological and immunohistochemical findings. Heat Shock Proteins (HSP 27, HSP 70, and HSP 90) were conducted on skin samples; anti-myoglobin antibodies were conducted on kidney and muscle tissue samples. There was widespread poly-visceral stasis. In the brain, edema and acute stasis were documented. The lungs had notable edema, acute stasis, and acute emphysema with septal rupture. The analysis of the skin demonstrated intense positivity to HSP 27 and HSP70. The muscles exhibited plenty of rhabdomyolysis and vacuolization of the muscle fibers. In the muscle and renal sample, tested with the anti-myoglobin antibody, intense positivity was demonstrated. The renal tubules were filled with myoglobin cylinders.

It is common to observe diffuse stasis and acute edema of the brain and the lungs, as well as the cutaneous positivity of HSP 27, HSP 70, and HSP 90. HSPs are proteins that are synthesized especially in response to various forms of cellular stress, such as high temperature, infections, free radicals, and biomechanical forces, to protect proteins from denaturation. In addition, HSP inhibits cell apoptosis induced by various stimuli, including the thermal stimulus, being detected early in case of exposure to heat. The expression of HSPs is related to the time of exposure to heat. Indeed, HSP 27 is the earliest to appear. The positivity to anti-myoglobin antibodies in muscle and the kidneys is also important to detect massive rhabdomyolysis.

#### Heat Shock Proteins, Hyperthermia, Anti-Myoglobin Antibodies



### H11 The Strange Trajectories of Death: Avtomat Kalashnikov—The New Weapon of the Garganic Mafia

Mauro A. Ciavarella, University of Foggia, Forensic Department, Foggia 71121, ITALY; Lorenzo Spagnolo, MD, Department of Forensic Pathology, Foggia, Foggia 71122, ITALY; Santina Cantatore, Foggia 71100, ITALY; Gianfranco Guccia, Palermo 90145, ITALY; Marcello Rendine, DBA, Department of Forensic Pathology, Foggia 71100, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY; Irene Riezzo, MD, PhD\*, University of Foggia, Foggia 71100, ITALY

**Learning Overview:** The goal of this presentation is to review wound ballistic evidence that comes from a new murder weapon used by the Garganic Clans (the Apulian Mafia in southern Italy): the Avtomat Kalashnikov (AK). This presentation focuses on macroscopic and histological findings used to distinguish between different weapon injuries, with particular regard to the wounds produced by the AK-47 rifle and AKM-74 missiles.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the need for a different approach to autopsy because of the high-velocity tumbling and fragmentation of AK bullets, describing their unusual trajectories in the body.

In the past few years, the Clans of the Gargano Mafia have radically changed their methods of murder. In fact, the methods of execution were characterized by the propulsion of multiple shots from firearms (shotguns), some of which exploded in the face, serving the double purpose of killing the victim and simultaneously scarring the body that would later be seen by relatives. However, in the past three years, they have changed “the murder tool”: in addition to the usual weapons, the AK is being used. The Garganic Clans use the AK not only for its high-offensive fire potential, but also because it is a symbol of power among the rival clans.

The AK is a gas-fired, selective assault rifle, developed in the Soviet Union, that comes from military scenarios. The original version (AK-47) of this weapon fires the 7.62mmx39mm cartridge with a muzzle velocity of 715m/s. The next version (AKM-74) of this weapon converted the rifle to the intermediate-caliber, high-velocity 5.45mmx39mm cartridge with a muzzle velocity of 900m/s. The different characteristics of these projectiles in comparison with other known firearms (shotguns, handguns), such as mass, caliber, velocity, shape, and material, explains different missile-tissue interaction.

The many and different types of weapons used for the “new” homicides, and the presence of multiple entrance wounds on the victim’s body (average  $\pm 35$ ), forced the use of a different methodological approach to the decedent. This study reports a summary of wound ballistic evidence from nine cases of homicide committed with multiple weapons, including the AK from the Garganic Clan’s wars. At the primary crime scene, ballistic elements were collected. Then, an external regional examination and radiological investigation (X-rays, Multi-Slice Computed Tomography (MSCT)) of the body were performed to detect the bullets.

The gross-examination of the head showed the AK bullets produced shattering of bone. The macroscopic form of the entrance wound of the AK bullet into soft tissues was not constant and depended on the presence of previous interactions with other objects (e.g., car doors). Likewise, the shape of the exit wound was not regular and depended on the injured tissues. When the AK47 bullet passed only through soft tissues, the instability characteristics of the 7.62mmx39mm bullet led to a net via the parenchymatous organs, with holes approximately 1.5cm in diameter. Extremely complex, non-linear, and multiple, spreading intracorporeal trajectories were observed. This phenomenon is due to the destabilization of the bullet during penetration (tumbling phenomenon) and its fragmentation into small and separate parts.

The histological examination of the entry and exit wounds and of the injured organs showed loss of tissue structure, cavitation, vacuolation, few red blood cells, perivascular ring hemorrhages, the presence of passive transport elements (muscular and cutaneous elements in liver), and foreign bodies. These histological findings are different from the lesions produced by low-velocity ammunition.

#### Avtomat Kalashnikov, Histological Findings, Tumbling



### **H12 Cardiac Wounds Induced by Cardiopulmonary Resuscitation in a Context of Phlebotomy: A Case Report and a Review of the Literature**

*Erwan Le Garff, MD\*, Institut Médico-Légal/Forensic Institute, Lille Cedex, Nord 59037, FRANCE; Vadim Mesli, MD, Institut Medico Legal, Lille Cedex, Nord 59037, FRANCE; Philippe Morbidelli, MD, Lille 59037; Anne A. Becart, DDS, PhD, Service De Medecine Legale, Lille 59000, FRANCE; Valéry C. Hedouin, MD, PhD, Lille, Hauts De France 59037, FRANCE; Didier Gosset, MD, PhD, Institut de Medecine Legale, Lille 59045, FRANCE*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### H13 Retinal Hemorrhages and Spinal Nerve Root Hemorrhages in Pediatric Resuscitated Near-Drownings: Recognizing Ischemia-Reperfusion Injuries

*Anna G. McDonald, MD\**, Wake Forest Baptist Medical Center, Winston Salem, NC 27157; *Nathan S. Shaller, MD*, Wake Forest Baptist Medical Center, Winston Salem, NC 27157; *Patrick E. Lantz, MD*, Wake Forest University, School of Medicine, Winston-Salem, NC 27157-1072

**Learning Overview:** The goal of this presentation is to demonstrate the extent of reperfusion injuries in the eyes, spinal cord, and central nervous system in two pediatric cases of resuscitated, near-drowning events with prolonged resuscitative efforts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how retinal hemorrhages and spinal nerve root hemorrhages are due to hypoxia-ischemia reperfusion injury and are not specific for inflicted trauma.

A complete and thorough autopsy is critical in a subset of accidental pediatric deaths where circumstances are uncertain. Reported here are two resuscitated, near-drowning events in young children who developed fatal hypoxic-ischemic brain injury with herniation, retinal hemorrhages, spinal cord ischemia, and spinal nerve root necrosis and/or hemorrhage. This presentation highlights the necessity of performing a meticulous examination in near-drowning events, including a fundal examination, brain examination, and spinal cord examination to include both nerve roots and dorsal root ganglia.

The first case is a 4-year-old girl who was found submerged in a residential pool at her grandmother's house after being seen one to two minutes prior. Her uncle pulled her from the pool and immediately began cardiopulmonary resuscitation. Emergency Medical Services (EMS) arrived and intubated her prior to her bradycardia and subsequent cardiac arrest with pulseless electrical activity. Resuscitation was performed for approximately one hour prior to the return of spontaneous circulation. Pupils at the time were fixed and dilated, and the child was transferred to a tertiary center where a head Computed Tomography (CT) scan showed diffuse cerebral edema. A CT scan of the chest and abdomen showed sternal fractures (secondary to resuscitation), pulmonary edema, and diffuse dilation of the small bowel. Her condition continued to decline, and she was pronounced brain dead two days after the immersion incident.

The second case is a 7-year-old boy who was found fully submerged in a community pool after being seen playing approximately three to four minutes prior. Video footage later showed the boy jumping in the pool and playing prior to falling beneath the surface of the pool. EMS arrived to find the boy pulseless and performed resuscitative measures for at least 30 minutes prior to the return of spontaneous circulation. On arrival to a tertiary care center, his pupils were asymmetrically dilated and non-reactive. A head CT scan showed diffuse cerebral edema and hypoxic ischemic injury. A CT scan of the chest showed acute non-displaced, bilateral rib fractures (consistent with resuscitation) and lung changes suggestive of aspiration and pulmonary edema. Two brain death examinations were performed prior to pronouncement of death approximately 3.5 days after the near-drowning. Permission was granted for organ procurement.

Autopsy findings for both cases showed diffuse cerebral edema, bilateral aspiration pneumonia, and softening of the spinal cord. The younger child also had bilateral optic nerve sheath hemorrhages. Postmortem monocular indirect ophthalmoscopy revealed retinal hemorrhages in varying numbers, with the younger child having bilateral retinal hemorrhages (10-15 right eye, 50 left eye) and the older having a single hemorrhage. Histologically, the spinal cord and brain of both showed hypoxic-ischemic changes, accompanied by hemorrhage in the lumbar cord of the older child. Sampled spinal nerve roots showed perineural and intraneural necrosis, with associated hemorrhage in the case of the older child. No additional injuries were identified that caused or contributed to the deaths.

Near-drownings with prolonged downtimes and subsequent resuscitation lead to diffuse hypoxic injury of the brain and spinal cord. While no antemortem fundal examinations were performed during the hospitalization, postmortem fundal examination revealed retinal hemorrhages not clinically expected. Meticulous examination of the spinal cord also showed each child had nerve root ischemic changes and hemorrhage, thought by some authors to be specific for a shaking mechanism of injury. These cases highlight the importance of fundal examination and spinal cord examination in the resuscitated pediatric population to detail the extent of the ischemia-reperfusion injuries throughout the nervous system.

#### **Retinal Hemorrhages, Spinal Nerve Root Hemorrhages, Hypoxia-Ischemia**



## H14 Only the Vagus Nerve: A Pair of Unusual Homicides

Stacey L. Reed, DO\*, Allison Park, PA 15101; Todd M. Luckasevic, DO, Allegheny County Medical Examiner's Office, Pittsburgh, PA 15222; Abdulrezak M. Shakir, MD, Allegheny County Medical Examiner's Office, Pittsburgh, PA 15222; Karl E. Williams, MD, Allegheny County Medical Examiner's Office, Pittsburgh, PA 15222

**Learning Overview:** The goal of this presentation is to increase awareness of an uncommon potential cause of death in the setting of penetrating head and neck trauma without massive injury.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a unique finding at autopsy and encouraging others to consider isolated vagal stimulation or injury as a precipitator of cardiac arrest and death.

The vagus nerve is the longest autonomic nerve in the body, providing parasympathetic control of the heart, lungs, and gastrointestinal tract. Running down the bilateral neck adjacent to the internal carotid arteries, it gives off a branch to the carotid body before entering the thorax and abdomen. Along with the glossopharyngeal nerve, it is responsible for regulating heart rate and blood pressure via the baroreflex, and thus can be implicated in instances of cardiac arrest due to neck trauma.<sup>1,2</sup>

Described here are two deaths in which violent actions (a gunshot and a stabbing) to the head and neck failed to illicit what would be considered “fatal” injuries; in each case, the vagus nerve was the only structure with any demonstrable trauma.

**Materials and Methods:** Case #1 is a 17-year-old African American male found lying in the street for an unknown interval with a gunshot wound to the head. Resuscitation efforts were unsuccessful, and the decedent was brought to the Allegheny County Medical Examiner's Office (ACMEO) as a homicide.

Case #2 is a 59-year-old Caucasian female who was stabbed twice in the neck with a knife by her husband. She was able to run to a neighboring home for help, but as Emergency Medical Services (EMS) was rendering aid, she went into cardiac arrest. Upon arrival at the hospital, emergent laryngoscopy and surgical wound exploration were performed, noting platysmal disruption, but all major vessels were intact. The patient never regained consciousness and after a short hospitalization was brought to the ACMEO as a homicide.

**Results:** Case #1—Examination of the head and neck revealed an indeterminate-range entrance gunshot wound to the right face, with the bullet lacerating only the skin, subcutaneous tissues and muscles of the face, and left lateral neck and contusing the left vagus nerve before exiting the left upper lateral neck. The vertebral bodies, mandible, skull base, and major blood vessels were undamaged.

Case #2—Examination of the head and neck revealed two stab wounds to the right lower anterior neck and left upper anterior neck, both lacerating only the skin, subcutaneous tissues, and muscles of the neck. All major blood vessels were spared; there was hemorrhage surrounding the right vagus nerve. Examination of the cervical spine, vertebral arteries, and spinal cord were unremarkable.

**Discussion:** A literature review for reports of violent deaths with only vagal trauma revealed a case of a screwdriver stab to the neck, which similarly spared all major neck structures, yet resulted in the patient's death.<sup>3</sup> Schrag et al.'s review of Cardioinhibitory Reflex Cardiac Arrest (or CiRCA) deaths found that of 48 potential CiRCA deaths reported between 1881 and 2009, only one death could not potentially be attributed to another cause.<sup>4</sup> While vagal stimulation is known in the anesthesia literature to be a potential cause of cardiac arrest, it is difficult to assign as a cause of death, as there are often many confounding factors.

If a thorough autopsy in a case of violence (e.g., shooting or stabbing) to the head and neck fails to reveal enough vascular, nervous, or bony trauma to account for death, the vagus nerve should be carefully examined for hemorrhage or contusion and considered as the cause of death.

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### Vagus Nerve, Homicide, Penetrating Head and Neck Trauma



### H15 Unusual Frangible Ammunition Used in a Homicide: A Case Report

*Sandra E. Jones, BS\*, Office of the Chief Medical Examiner, Raleigh, NC 27607; Michelle B. Aurelius, MD, Office of the Chief Medical Examiner, Raleigh, NC 27699-3025; Chad Moose, Rowan County Sheriff's Office, Salisbury, NC 28144*

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**Learning Overview:** After attending this presentation, attendees will better understand frangible projectiles and how ballistic testing from law enforcement can identify frangible projectiles, confirm the number of gunshot wounds, and complement autopsy findings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the need for the forensic pathologist to be familiar with frangible projectiles that are no longer in production as well as how law enforcement ballistic testing can support autopsy findings.

Frangible projectiles for firearms are designed to fragment upon impact. They are mainly used for training purposes and can provide improved safety in some situations by reducing the risk of ricochet and overpenetration of secondary targets. Frangible bullets are made of composites of hybrid materials and can produce atypical injury patterns at autopsy. In addition, the projectile fragmentation can make it difficult to determine the number of gunshot wounds at autopsy, with multiple projectile fragments with their associated multiple wound tracks from a single gunshot wound. The forensic pathologist must be familiar with the evolving firearm and ammunition industry, including knowledge of frangible projectiles that are growing in popularity with manufacturers.

This presentation describes a case of a 62-year-old African American female who was found dead in her home with gunshot wounds of the head. At the scene, law enforcement found three 9mm shell casings. Radiographs showed nine radiopaque projectiles in the head and neck. Two gunshot wound defects were identified on the head. Mild to moderate decomposition with diffuse postmortem softening of the brain and one of the entrance gunshot wounds being atypical (internal and external beveling, no discernible marginal abrasion) made gunshot wound evaluation difficult. Ultimately, two entrance gunshot wounds of the head were identified at autopsy and nine projectile fragments were recovered. Five fragments were spoon-like, convex on one side with a shallow bowl on the other. Two fragments consisted of bases, and the additional two fragments were relatively small. None of the objects appear to have exited.

Law enforcement identified a suspect and it was revealed that Aguila® “IQ” 9mm hollow point ammunition was used during the shooting. The Aguila® “IQ” ammunition has been out of production for approximately a decade. It was produced in Mexico and is not a commonly used projectile. It was designed to break into three or four pieces when it struck a firm surface.

Law enforcement obtained a box of this ammunition and test fired it into a tank of water. Four rounds were test fired, and each round broke into four pieces, resulting in a base and three leaves.

Assessment of the fragments recovered at autopsy and correlation with the law enforcement ballistic findings support that one leaf fragment exited the atypical gunshot wound of the forehead, as supported by a keyhole defect in the skull. The remaining projectile fragments recovered were consistent with the fragments obtained from the decedent, supporting that each frangible projectile fragmented in a similar manner, with three leaves and a round base. The number of fragments found is consistent with two “IQ” ammo projectiles entering the head and one leaf fragment exiting the head.

This case emphasizes the wound interpretation challenges at autopsy that can arise with frangible ammunition as well as the importance of the utilization of firearm ballistics to correlate with wounds.

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#### Gunshot Wound, Ballistics Testing, Frangible Ammunition



### H16 Plugged, Unplayable Lies: A Case Report of a Novel Suicide by Foreign Body Airway Obstruction

Stacey L. Reed, DO\*, Allison Park, PA 15101; Todd M. Luckasevic, DO, Allegheny County ME, Pittsburgh, PA 15222

**Learning Overview:** The goal of this presentation is to illustrate a novel case of suicide by asphyxiation wherein the decedent intentionally self-occluded the airway by the introduction of multiple foreign objects.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the need for thoughtful and thorough scene investigation regarding manner of death in a case that could be interpreted as a likely homicide, as well as by highlighting the inventive nature of suicide enactors.

**Introduction:** Asphyxial deaths by choking and airway obstruction are most often characterized as accidents, such as elderly or inebriated individuals choking on a food bolus, or homicides, as in the case of robbery victims being silenced by gags.<sup>1</sup> Rarely are there reports of intentional self-introduction of foreign objects into the larynx for the purpose of taking one's own life. Such a case is described here.

**Materials and Methods:** The decedent was a 45-year-old Caucasian male found dead one morning in the basement of the home he shared with his mother. There were three strips of white tape measuring 1½ inches wide over the decedent's mouth, and there were multiple additional strips of tape hanging from the side of a nearby table. He was last known to be alive eight hours prior, and there had been no indication of intent for self-harm at that time. The scene showed no sign of a struggle or foul play, and there was no evidence of autoerotic behavior. The decedent had no known psychiatric history or evidence of recent psychosis.

**Results:** At autopsy, the decedent was found to have three soft-sided foam golf balls, each measuring 4cm in diameter, deep in the oral cavity occluding the larynx, with strips of adhesive tape fully covering the mouth. There were healing superficial incised wounds of the bilateral anterior wrists. There was no other evidence of acute trauma or recent violence. The remaining examination was unremarkable. Postmortem toxicology performed on blood was negative. The cause of death was determined to be asphyxiation due to airway obstruction by foreign bodies.

Upon further investigation, it was revealed that the decedent was about to be incarcerated for federal felony charges and had made comments to the effect that he "would not be going to jail." The manner of death was determined to be suicide.

**Discussion:** A literature review for suicidal self-asphyxiation with foreign bodies revealed these cases to be extremely rare, with most decedents choking on paper products or cloth.<sup>2-4</sup> The rarity of suicidal self-asphyxiation is likely due to the act being difficult to complete.<sup>5</sup> Unlike this current case, the actors typically demonstrate some degree of psychosis or have a history of schizophrenia.

Determining the manner of death can be challenging when the cause of death seems violent or unusual. As always, thorough scene investigation is crucial to be able to parse out suicidal intentions from what would otherwise appear to be accidental or even homicidal in nature.

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2. Zhang L., Guo Y., Chen S., Cai J. Custody Suicide With Washrag: A Case Report With Clinical and Custodial Consideration. *Journal of Forensic Legal Medicine*, 2013 (May); 20(4): 343-344.
3. DeDonno A., Marrone M., Santoro V., Ostuni A., Cassano A., Grattagliano I., Introna F. A Singular Case of Asphyxia by Choking on a Handkerchief: Accidental Event or Suicide to "Shut Up" Spirits. *Clinical Therapeutics*, 2017 (September-October); 168(5): e293-e296.
4. Sauvageau A., Yesovitch R. Choking on Toilet Paper: An Unusual Case of Suicide and a Review of Literature on Suicide by Smothering, Strangulation and Choking. *American Journal of Forensic Medicine and Pathology*, 2006 (June); 27(2): 173-174.
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#### Suicide, Asphyxia, Airway Obstruction





### H17 Death by Fractal Art: A Tale of Beauty and Electrocutation

*Tasha Zemrus Greenberg, MD\**, Tarrant County Medical Examiner's Office, Fort Worth, TX 76104-4919; *Nizam Peerwani, MD*, Tarrant County Medical Examiner's Office, Fort Worth, TX 76104-4919; *Stephen P. White*, Tarrant County Medical Examiner's Office, Fort Worth, TX 76104

**Learning Overview:** After attending this presentation, attendees will understand the importance of a thorough scene investigation in determining the cause of death in cases of suspected electrocution.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating medical examiners and forensic investigators about the features of deaths by electrocution and, specifically, the dangers involved in fractal art, as well as stressing the necessity of a thorough investigation of the scene to aid in the determination of cause of death.

On March 19, 2018, a 50-year-old male with a history of hypertension and methamphetamine use was found unresponsive in the garage at a private residence. He was taken to a local hospital where he was pronounced. The Tarrant County Medical Examiner's Office was notified of this death by a nurse at the hospital due to suspected electrical burns.

On March 20, 2018, at 11:00 a.m., the forensic investigator met with the decedent's mother. The decedent had been found supine near a homemade wood-burning craft machine plugged into an electrical socket, with a jumper cable clamp near his right hand and another lying across his chest. The mother had received a shock when she tried to remove the device and had thrown it into a trash bag, which was retrieved. The decedent had made the device himself for the hobby of wood burning. No water was seen on the concrete floor near the decedent.

The device was known as a "Lichtenberg Fractal Art wood-burning device," fashioned from a pair of six-foot-long jumper cables that were attached to what appeared to be a transformer out of a microwave oven that had a label stating: "JMOT-N 60A2-61T 120V/60Hz JI YUNG SA CO LTD # 9905111 DANGER HIGH VOLTAGE." The transformer was used to increase the power output to high voltage electricity. The handles of the jumper cables were wrapped in black electrical-type tape, with one of the cable's clamps having a bent/scorched nail in its teeth. The transformer had attachment points that were soldered on, then attached to a section of three-pronged electrical cord marked "14 AWG 12.09mm 105 deg C 300 V VW-1 -- LINETEK—" on the rubberized shielding. The device had been plugged into an older-style toggle switch power strip mounted to a wall. The power strip was found marked "MAX TOTAL RATING 12AMP 125V AC/DC." All the electrical breakers were on, had not tripped, nor had they been reset.

The decedent had been making fractal art for a few months and there were multiple examples in the garage. The art is created by making a circuit in the wood and using a slurry mixture of baking soda mixed with water; the water is used to complete the circuit and the baking soda to control the extent of the burn.

On July 24 2017, as reported in *Woodworking Industry News*, The Safety Committee of the American Association of Woodturners issued a policy against fractal burning, banning it from the American Association of Woodturners (AAW) -sponsored events.<sup>1</sup> This came after it was reported that in May 2017, an experienced woodworker in Walla Walla, WA, was electrocuted while making a picture frame using the Lichtenberg fractal burning technique. The coroner said the process was very dangerous.

Findings at autopsy included pulmonary congestion and edema, cerebral edema, mild fatty metamorphosis of liver, and thermal injuries of the chest with multiple deep troughs with blackened rims across the chest, present in a discontinuous, obliquely oriented line from the right upper to left lower chest. There was burning of the palmar surface of the right index finger extending toward the thenar eminence. There was also a faint area of brown discoloration on the sole of the left foot near the heel, suggestive of burn injury. Toxicology was positive for methamphetamine and amphetamine. The cause of death was ruled high voltage electrocution and the manner of death accident.

This case illustrates not only the danger of the fractal woodworking technique with regard to electrocution, but also the importance of thorough scene investigation to ensure an understanding of the circumstances surrounding the death and to ensure an accurate ruling of cause and manner of death.

**Reference(s):**

<sup>1</sup>. Sampson, W. Death Prompts Ban on Fractal Burning, *Woodworking Industry News*, July 24, 2017.

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**Fractal Art, Electrocutation, Wood Burning**



### H18 Homicidal Violence Against Children in Mississippi: Four Case Reports

*Mark M. LeVaughn, MD\**, Mississippi State Medical Examiner's Office, Pearl, MS 39208; *L.R. Funte, MD*, Mississippi State Medical Examiner's Office, Pearl, MS 39208; *Brent Davis, MD*, Mississippi State State Medical Examiner's Office, Pearl, MS 39208; *Anastasia Holobinko, PhD*, Mississippi State Medical Examiner's Office, Pearl, MS 39208; *Steven A. Symes, PhD*, Mississippi State Medical Examiner's Office, Pearl, MS 39208

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**Learning Overview:** After attending this presentation, attendees will understand the variation observed in homicidal cases as it pertains to violent crimes against children in the state of Mississippi. According to recent statistical data, Mississippi exhibits the highest infant mortality rate in the United States; however, these mortality rates do not include child deaths resulting from repeated abuse, neglect, or random criminal acts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing four cases of homicide in which the violence or maltreatment was perpetrated against children aged 5 to 13 years by parents or other relatives, non-parents (e.g., acquaintances or partners of relatives), or strangers.

**Case 1:** The partially clothed body of a 5-year-old female was found in an unoccupied mobile home by search teams the day after her abduction from her apartment complex. The postmortem examination revealed evidence of blunt trauma to the face and extremities and penetrating peri-genital injuries indicative of sexual battery. Dependent lividity and regionalized petechiae were consistent with asphyxia due to ligature strangulation (hanging). A male resident/visitor at the complex was eventually arrested and charged with capital murder. He was convicted and sentenced to death.

**Case 2:** A 7-year-old Black male and his mother were reported missing in late 2013. Their overturned and burning car was discovered, and their bodies and that of the child's stepfather were found inside an abandoned house. The juvenile's cause of death was identified as a single shotgun wound of the right forearm into the back with injury to the upper extremity and thoracic region. Authorities believe that the stepfather was killed first, and the child and his mother were subsequently taken to the house and shot. The perpetrator was convicted on two counts of capital murder.

**Case 3:** The decedent was an 11-year-old male who was found unresponsive in the bathroom of his father's and stepmother's home, and subsequently declared dead at the scene. Pertinent findings at autopsy were emaciation with blunt force injuries of the head, neck, torso, and extremities of sufficient severity to cause death. Body weight and organ weights were abnormally low, and subcutaneous adipose tissue and mesenteric fat were scant. The stepmother received a life sentence for second-degree murder; the boy's father was sentenced to 40 years in prison.

**Case 4:** This 13-year-old female and her mother were victims in a domestic murder-suicide carried out by the mother's live-in boyfriend. All three bodies were found at the residence by authorities after efforts to contact them had failed. The child died as a result of a close-range perforating gunshot wounds to the hands and forehead.

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### Homicide, Children, Child Abuse



### H19 What a Dangerous Place: An Unusual Homicide in the Hospital

Roberto Vaglio, via Napoli n.2, Nardò; Alessandro Dell'Erba, PhD, Risk Management Unit, Bari 70124, ITALY; Francesca Donno, MD\*, University of Bari, Bari 70121, ITALY

**Learning Overview:** After attending this presentation, attendees will understand patient outcomes resulting from a complex mix of preventable and/or unpreventable factors.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that an absolutely safe place doesn't exist, and autopsy is an essential investigative procedure.

Presented here is a strange case of murder: a hospital—a health care institution providing patient safety—became the setting for an act of lethal aggression.

A 73-year-old woman, confined to bed after a fracture of the femur, suffering from severe Alzheimer's disease and spasticity post cerebral ictus, was admitted to the emergency department with reported episodes of hypotension and bradycardia. After a neurological assessment, the woman was waiting for the diagnostic results in the waiting room. There, from 3:53 a.m. to 3:54 a.m. (when her son, who is assisting her as a caregiver, leaves her for a moment to go to the bathroom), she is the victim of an aggression by another patient (male, 42 years old, repeat offender with psychic disturbances, not in therapy, who had gone to the emergency room for a reported aggression by a known person).

The postmortem examination showed the right temporal region had an excoriated ecchymosis, irregularly oval, with a red-violet color, with small epidermal ridges, with a full thickness discontinuity in its center of the galea capitis, irregularly quadrangular, with dimensions of 2mm x 5mm. The soft tissue wound was next to a hole in the bone of a coarsely roundish shape, with neat margins, regular on the outside, approximately 7mm diameter, flared inside. The described wound, which calls to mind the entrance hole of a single projectile, had a more or less circular shape on the bone that reproduced the shape of the harmful tool. The dura mater presented a wound with morphological characteristics and dimensions compatible with the use of a tool such as a screwdriver, with an approximate diameter of 7mm. There was also a significant presence of small bone fragments in the brain parenchyma, transported inside by the action of the tool.

Penetrating head injuries due to the use of screwdrivers as wounding agents in acts of interpersonal violence are rarely reported events in forensic literature, and the mortality rate is approximately 47.6%.<sup>1</sup>

The cause of death in the present case report was a cardio-respiratory arrest by massive intra-parenchymal hemorrhage, widespread subarachnoid, and bilateral hemispherical subdural (as often happens with unipolar trauma) because of traumatic penetration of the skull.

The thickness of the bone of the victim—not particularly consistent—had facilitated the penetration of the tool and, therefore, the depth of the damage (determined by the introduction of the shaft inside the cerebral parenchyma), different than what has happened after a similar action of the same tool (screwdriver) in well-known cases in the literature.<sup>2</sup>

The autopsy allowed the reconstruction of the dynamics of mortal wounding and of the modality/tools used, even if there were no documented probative-value elements (activated cameras in the waiting room). The probative value of other evidence confirmed the reconstruction. In cases in which technology has its limits (malfunction of the video surveillance system of the waiting room), the integrated evaluation of the acquired elements with the medicolegal investigation can be successful: the compatibility of the injuries found with the use of a rigid tool, as a screwdriver (cut or star pattern), used as a dagger, led to the murderer.

In conclusion, the case presented will demonstrate that the solution is teamwork and an accurate integration of each element of probative value.

#### Reference(s):

1. Pavlidis P. et al. Traumatic Brain Injury Due to Screwdriver Assaults: Literature Review and Case Report. *Am J Forensic Med Pathol.* 2016 Dec; 37(4):291-298.
2. Purtskhvanidze K. et al. Orbital Screwdriver Injury in a Toddler, *Ophthalmologie.* 2018 Jan 24. Doi: 10.1007/s00347-018-0654-8.

#### Hospital Murder, Screwdriver, Penetrating Head Wound

### H20 The “Tin Man”: Another Mafia Homicide

Francesca Donno, MD\*, University of Bari, Bari 70121, ITALY; Alessandro Dell’Erba, PhD, Risk Management Unit, Bari 70124, ITALY; Eloisa Maselli, MD, Bari 70122, ITALY; Roberto Vaglio, via Napoli n.2, Nardò, ITALY

**Learning Overview:** After attending this presentation, attendees will understand the importance of an accurate “investigation” to interpret Mafia-related crimes. The murders committed and/or commissioned by a criminal organization are characterized both by method used and positioning of the body with a characteristic admonitory significance.<sup>1-3</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as an updated attestation of how the Mafia has preserved its own homicidal modality in the past years; a complete explanation of this will be provided only by careful observation and analysis of present evidence.

A metal-sheet drum covered with branches was found in pine trees close to a metropolitan area in southern Italy. The drum contained a male corpse in a fetal position, covered with stones, soil, concrete, an empty red-colored plastic bottle of hydrochloric acid, and a small “free” rope of approximately 1 meter in length. There was no clothing except for a small piece of fabric around the neck and two dark-colored socks. No identifying features of the face were recognizable. The skin was grayish-black, friable, and inelastic.<sup>4</sup> The hypostases were not visible, and the cadaveric rigidity was artefactual.

The autopsy showed a complex pattern of skeletal lesions, preliminarily demonstrated by total body Computed Tomography (CT) as follows: a fracture with multiple fragments of the lateral wall of the left orbit, disarticulation of the temporal process of the zygomatic bone, depressed, multiple fragment fractures of the left temporal bone, fracture of the lateral wall of the right orbit, and fracture of the maxillary bone with absence of some front teeth (incisors and canines). The mandible was disarticulated from the maxillofacial bones.

There was advanced liquefaction of the tissues of the neck: the head was connected to the trunk only through tendon and slender muscle-cutaneous flaps with dislocation of the anteroposterior axis of about 45° C5 on C4 and C7 on C6. The histological analysis showed some areas of subdural hemorrhage. The study of the other organs and viscera, despite the advanced liquefaction and putrefaction, excluded alternative causes of death.

The described injuries are due to blunt head and maxillofacial trauma. The morphology of the skeletal lesions, particularly in the left temporal region, is typical for the contusive nature of the fractures, produced with an object that probably did not have a large surface (stick, fist, stone, other). The left temporal region, the orbits, and the buccal region presented high-energy fractures caused by a force almost completely perpendicular to the surfaces. The site of the lesions suggests that the victim and the aggressor faced each other.

The analysis of molecular genetics, including the study of the polymorphisms of DNA extracted from the buccal swab of the two presumed sisters of a deceased subject and from the cadaver’s tooth, allowed this study to confirm the sharing of both parents with a probability equal to 99.99999999% (LR=4.21e + 15) with the consequent identification of the “tin-man.”

In conclusion, the case presented will demonstrate that these Mafia-style homicidal methods preserve a timeless symbolism representing a typical mark of belonging.

#### Reference(s):

1. Pomara C. et al. “Lupara Bianca” A Way to Hide Cadavers After Mafia Homicides. A Cemetery of Italian Mafia. A Case Study. *Leg Med* (Tokyo). 2015 May;17(3):192-7. doi: 10.1016/j.legalmed.2014.12.008. Epub 2014 Dec 30.
2. D’Errico S. et al. A Novel Macabre Ritual of the Italian Mafia (‘Ndrangheta): Covering Hands With Gloves and Burying the Corpse With Burnt Lime After Execution. *Am J Forensic Med Pathol*. 2011 Mar;32(1):44-6. doi: 10.1097/PAF.0b013e3181efbbf2.
3. De Donno A. et al. Manners of Killing and Rituals in Apulian Mafia Murders. *J Forensic Sci*. 2009 Jul;54(4):895-9. doi: 10.1111/j.1556-4029.2009.01068.x. Epub 2009 May 26.
4. Kozawa S. et al. An Autopsy Case of Chemical Burns by Hydrochloric Acid. *Leg Med* (Tokyo). 2009 Apr;11 Suppl 1:S535-7. doi: 10.1016/j.legalmed.2009.01.008. Epub 2009 Mar 6.

#### Tin Man, Mafia Murder, Genetic Investigation



### **H21 An Autopsy Case of Suicide With Three Knives: A Forensic and Anatomical Discussion**

*Beatrice Defraia\**, Florence, ITALY; *Martina Focardi*, Florence 50134, ITALY; *Niccolo Norberti*, Florence, ITALY; *Stefano Colagrande*, Florence, ITALY; *Vilma Pinchi*, PhD, University Section of Forensic Medical Sciences, Florence, Tuscany 50125, ITALY; *Gian A. Norelli*, PhD, University Section of Forensic Medical Sciences, Florence 50125, ITALY

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**THIS ABSTRACT WAS NOT PRESENTED.**



### H22 An Analysis of Risk Factors Involving All-Terrain Vehicle (ATV) Deaths

Charlene Admissions, BA\*, Western Michigan University School of Medicine, Kalamazoo, MI 49008; Joanne Catania, MS, Western Michigan University School of Medicine, Kalamazoo, MI 49008; Theodore T. Brown, MD, Kalamazoo, MI 49008

**Learning Overview:** After attending this presentation, attendees will better understand the categorized risk factors of ATV-related deaths, including modifiable risk factors that could potentially reduce the mortality rate of ATV-related deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by re-approaching medical examiner investigator reporting on modifiable risk factors relating to ATV deaths, creating a more consistent database for factors that can influence ATV-related deaths.

Each year, there are approximately 650 deaths and 100,000 injuries that involve ATVs.<sup>1</sup> Recognized risk factors of ATV-related accidents and deaths include not wearing a helmet, riding with more than the recommended number of riders, adequate training, driving on paved roads, and young age of the driver.<sup>1</sup> This significant number of ATV-related injuries and deaths warrant an even more detailed investigation of risk factors. This study presents a series of 11 ATV-related deaths in western Michigan where an investigation and/or postmortem examination was performed in order to review the circumstances of individual ATV-related deaths and identify modifiable and non-modifiable risk factors.

Of all ATV-related deaths reviewed, 10 of the 11 decedents were male (91%). The manner of deaths was all accidental (91%), except for one (9%), which was a suicide where a male was found dead in a closed garage with a running ATV. Most commonly, the decedent was the driver (64%), all deaths occurred on ATVs while performing non-work-related activities, and the cause of death was most frequently due to blunt force injuries (64%). Seven decedents (64%) were found ejected from the ATV, two of which were found with the ATV on top of them, which is suggestive that seatbelts were not used. All but two deaths occurred off paved roads, which while this is a United States Consumer Product Safety Commission recommendation, the-off road locations increase the risk of poorer visibility and are potentially more difficult to navigate terrain. In addition, most deaths occurred early in the morning or late in the evening, which is suggestive of decreased visibility as a contributing factor to ATV-related deaths. Of note, the ages of decedents range from 11 years to 82 years (mean age of 39 years) and the majority of cases (55%) had no evidence of alcohol or illicit drugs at the time of death, which is suggestive that age and alcohol/illicit drugs are not strong indicators for increased risk of ATV-related deaths.

This study was most interested in identifying modifiable risk factors of ATV-related deaths, such as use of seatbelts and helmets, in order to reduce ATV-related deaths in the future. While Medical Examiner Investigators (MEI) often included this information in their written report or demonstrated in photographs at the scene, the information provided was not consistent. Therefore, this presentation suggests an additional checklist of specific ATV-related investigative information that could have potential impact in reducing future ATV-related deaths: (1) safety measures of ATV and ATV occupants (seatbelt availability; evidence of seatbelt use; protection around cabin (ex: roof, roll bars, and windshield); evidence of helmet use (if so, location and type of helmet); number of occupants involved in accident; experience and training of driver; known alcohol/drug use of occupants; and significant underlying natural diseases of occupants); (2) circumstances of crash (time; level of visibility; crash location (off-road versus paved road, groomed trail versus ungroomed area); type of crash; location of decedents; and extent of damage to ATV); and (3) ATV-specific information (make; model; horsepower/torque; passenger capacity; and sit versus straddle).

#### Reference(s):

<sup>1</sup> United States Consumer Product Safety Commission Infographic. *ATV Deaths & Injuries*. <https://onsafety.cpsc.gov/blog/2018/05/18/cpsc-infographic-atv-deaths-injuries/>. Accessed July 26, 2018.

#### ATV, Deaths, Safety



### H23 Delayed Subdural Hematomas in Decedents on Anticoagulation Medication in the County of Santa Clara, California

Michelle A. Jorden, MD, Santa Clara ME, San Jose, CA 95128; Karin E. Wells, BA\*, Aptos, CA 95003

**Learning Overview:** The goal of this presentation is to discuss several delayed subdural hematomas observed in a variety of manners of death at the Santa Clara county medical examiner/coroner's office from 2016 through mid-2018.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how decedents who are on anticoagulants and suffer a head injury do not necessarily present immediately with a subdural hematoma, which was also similar to the cohort not on anticoagulation. This finding underscores that a delayed subdural hematoma in these patients must be considered by the medical examiner as cause and manner of death may be affected.

The medical examiner/coroner's office has observed that decedents taking anticoagulant medication are not immediately presenting with evidence of traumatic brain injury in a variety of manners of death. Anticoagulation therapy is used for a multitude of medical illnesses, and the purported consequences of taking anticoagulation when sustaining a head injury are well established. While anticoagulant medications, such as warfarin, work to block the factors responsible for the coagulation of blood, there have been noticeable delays between injury and the presentation of symptoms of subdural hematoma. This poses a particularly interesting problem for medical examiners when rendering cause and manner of death, as traumatic brain injuries in anticoagulated patients may not always be acute. According to a study conducted by Rust et al., patients on warfarin are 42.5 times more likely to develop a chronic subdural hematoma.<sup>1</sup>

A retrospective search of the Santa Clara county medical examiner/coroner's database was performed for the years 2016 through mid-2018 with key words "subdural hematoma" and/or "blunt head trauma" encompassing all manners of death. The search resulted in 127 cases. The goal was to analyze basic demographics, time of injury to clinical presentation if known, and whether the decedent was on anticoagulation. One hundred five total accidental deaths (85 of which were accidental falls and 20 motor vehicle accidents), six homicides, eight undetermined, and eight natural deaths were identified. Of the 127, 38.5% of the decedents were on an anticoagulant medication, including warfarin, aspirin, clopidogrel, apixaban, rivaroxaban, ticagrelor, and one unknown. The most common anticoagulant medication was warfarin at 55%. The age range for all decedents in this study, whether on anticoagulation or not, was 5 months to 104 years of age, with average age at death being 71 years. The average age of decedents on anticoagulant medication was 81 as opposed to an average age of 65 for those not on anticoagulation. Of the 127 cases, 73% of individuals presented within the first 24 hours (range of minutes to 19 hours), whereas 27% of individuals presented after 24 hours; in these cases, clinical symptoms were more apparent (headache, vomiting, sleeping). In 77 cases in which exact time intervals from injury to clinical presentation were known, the average was 1.8 days.

The greatest percentage of deaths (68.5%) were related to accidental falls relating to traumatic head injuries. Of the 49 decedents on anticoagulant medications, 48 experienced an accidental fall; the average age at death for these decedents was 81 years. Of the 47 decedents who experienced falls while on anticoagulants, the average time from injury to clinical presentation among these decedents was 2.5 days. The reported timeframe corresponds with that presented by Volans, whose clinical study found that patients presented with complaints of headache up to three days after experiencing a fall.<sup>2</sup> There were an additional nine decedents for whom specific times to clinical presentation were not captured. Also of interest were three decedents who experienced a fall and had negative Computed Tomography (CT) scans initially; however, scans in the following days demonstrated subdural hematomas.

Of the six homicide cases, one decedent was taking anticoagulant medication and suffered craniocerebral injuries due to blunt head trauma. Four of the six homicide cases had thin subdural hematomas documented in addition to craniocerebral injuries, and all were pronounced deceased at scene.

Research on the delayed presentation of subdural hematomas in patients taking anticoagulant medication is limited, particularly from the perspective of the medical examiner.

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1. Rust T, Kiemer N, Erasmus A. Chronic Subdural Hematomas and Anticoagulation of Anti-Thrombotic Therapy. *Journal of Clinical Neuroscience*. 13 (2006): 823-827.
2. Volans A.P. The Risks of Minor Head Injury in the Warfarinized Patient. *Journal of Accident and Emergency Medicine*. 15 (1998): 159-161.

#### Subdural Hematoma, Anticoagulation, Delayed Clinical Presentation



### H24 Forensic Analysis in Mass Disasters: Were Gunpowder Explosions in a Fireworks Factory an Accident or a Terrorist Bombing?

*Francesca Tarantino, MD, Bari 70124, ITALY; Maricla Marrone, MD, Bari 70124, ITALY; Valentina Ronco, MD\*, Section of Legal Medicine D.I.M., Bari 70124, ITALY; Paola Corsignano Carrieri, Section of Legal Medicine D.I.M., Ballistic Centre, Bari 70124, ITALY; Stefania Lonero Baldassarra, Bari 70124, ITALY; Alessandro Dell'Erba, PhD, Risk Management Unit, Bari 70124, ITALY; Francesco Vinci, MD, Bari 70124, ITALY*

**Learning Overview:** After attending this presentation, attendees will understand more about the relevant role of forensic investigation and genetic analysis for the reconstruction of prior and consequential events in mass disasters.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing through an unfortunate event that explosions and/or bombings remain the most common deliberate cause of disasters involving large numbers of casualties, especially as an instrument of terrorism.

To address the problem to the maximum benefit of mass disaster victims, organization must be developed in classifying corpses, obtaining biological samples, and identifying people. The goal of this study is to evaluate the impact of different approaches to victims and to fulfill a valid protocol for their identification, which may gain importance in the resolution of forensic cases.

Reported here is the case of a fireworks factory in which combustible chemicals caused explosions and fires with consequent destruction of the factory and the death of all the workers. Four of ten victims had a meaningful Total Burn Surface Area (TBSA) of 99% and the last ones died in the hospital from cardiovascular collapse resulting from severe hypovolemic shock and sepsis. Two victims died from carbonization and the other four workers had carbonization associated with fractures of limbs (lower > upper), lacerations, amputations, decapitation, and massive body trauma resulting from explosions. The bodies of seven victims showed prominent traumatic changes that made it impossible to do individual identification, particularly for the mangled corpses and some that had extensive limb amputations and/or decapitation. A team performed autopsies and created a protocol to obtain biological samples (bones, blood, teeth, muscles). The forensic pathologists contacted the families of the alleged victims and each of them gave a blood sample collected for comparing DNA. The geneticist, using the method of extraction and gene amplification, obtained the DNA from bones, teeth, muscles, or blood samples taken from the victims, then compared with the those extracted from blood samples of the relatives; electropherograms showed at least one allele for each genetic marker of the Combined DNA Index System in common between the victims and the families, thus allowing the certain identity of everyone involved in the event. Therefore, it was possible to correlate the human remains to a specific person, then recompose the extensively traumatized body. After establishing the identity of all workers, it was possible to determine their position in the environment at the time of the occurrence of fires and explosions and, in this way, also identify the exact place of the first explosive event, which then, as a chain reaction, involved all the factory buildings containing gunpowder.

The results of the different forensic analyses (autopsies, genetic investigations, and traumatological ones) have made it possible to validate a scientific method useful in all mass disasters, even when it is difficult to do any type of anthropological investigation or forensic odontology. The reported case demonstrates how an accidental catastrophic event can be easily confused with a terrorist attack, the latter event not difficult to achieve especially, in the wake of current international events. Therefore, it is essential that in all events involving a large number of people, there is a highly specialized team in forensic fields that can easily recognize the nature of the traumatic event and direct further investigations useful to understanding the detrimental dynamics of mass disasters.

#### Mass Disasters, Genetic Analysis, Forensic Investigation





### H25 Small RNA Sequencing and Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) Validation of Forensically Relevant Body Fluids

Karly L. Johannsen\*, University of New Haven, West Haven, CT 06516; Claire Glynn, PhD, Forensic Science Department, West Haven, CT 06516

**Learning Overview:** The goal of this presentation is to inform attendees of several microRNAs (miRNAs) discovered via next generation sequencing that have the potential to be discriminatory for the identification of forensically relevant body fluids, including venous blood, semen, saliva, vaginal fluid, and menstrual blood.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the methodology of using next generation sequencing to discover known and potentially novel miRNAs that are specific to forensically relevant body fluids. Additionally, the forensic science community will have the opportunity to learn more about the validation of next generation sequencing results via RT-qPCR.

Body fluid identification is an important aspect of forensic investigations as it can assist with the reconstruction of a crime scene and can refute and/or support witness statements. Currently, there is no universal method for body fluid identification. Each body fluid has several tests, both presumptive and confirmatory. Additionally, there are no reliable confirmatory tests for the identification of menstrual blood and vaginal fluid. This causes body fluid identification to be a time-consuming process that can be highly destructive to an unknown body fluid sample. A universal method for body fluid identification that is sensitive, specific, efficient, and minimally destructive is necessary.

In recent years, miRNAs have been heralded as novel biomarkers for the identification of body fluids. They represent an ideal candidate for this purpose as they are remarkably stable, require minimal starting material, and can be co-extracted with DNA, thereby providing both body fluid identification and person identification simultaneously. Several published studies have reported various lists of miRNAs that show potential for the identification of particular body fluids. However, there is little agreement across the studies of which miRNAs are suitable, and few studies included menstrual blood and vaginal material. In addition, all miRNAs reported to date are shown to be differentially expressed across all body fluids, with none yet reported to be uniquely expressed in one body fluid. With the advent of Next Generation Sequencing (NGS), it is now possible to sequence all forensically relevant body fluids for both known and novel miRNAs, with the expressed interest to identify panels of miRNAs for each body fluid, ideally with several that are uniquely expressed in a particular body fluid.

The first goal of this study was to sequence a range of forensically relevant body fluids to identify known miRNAs and potentially discover novel miRNAs that are specific to venous blood, semen, saliva, menstrual blood, and vaginal fluid. The second goal of this study was to select panels of miRNAs, based upon the sequencing data, for RT-qPCR validation, that show promise for body fluid identification.

Following Institutional Review Board (IRB) approval, venous blood ( $n=10$ ), semen ( $n=5$ ), saliva ( $n=10$ ), menstrual blood ( $n=10$ ), and vaginal fluid ( $n=10$ ) was collected from volunteers with informed written consent. Each body fluid was extracted using the miRNeasy® miRNA isolation kit, following the manufacturer's protocol. Liquid fluids (venous blood, semen, saliva) were extracted using 500µL of each. Menstrual blood and vaginal material were collected on sterile cotton swabs and were therefore extracted directly from the swabs. Following miRNA isolation, the extracts were stored at -20°C until required. Four quantification methods were utilized, including the NanoDrop™ One<sup>C</sup> UV-Vis Spectrophotometer, the Qubit® 3 Fluorometer using the RNA HS Assay kit, and the Agilent® 2100 Bioanalyzer using both the Small RNA kit and the Nano 6000 kit. The concentration of each sample was obtained, and the integrity of the samples were analyzed in the form of the RNA Integrity Number (RIN). Samples were then prepared for sequencing using the TruSeq® Small RNA library preparation protocol. The prepared samples were then sequenced using the MiSeq® FGx platform. The sequencing data was analyzed using the Illumina® BaseSpace small RNA app. Several miRNAs were chosen for further RT-qPCR validation, based on their potential applicability for body fluid identification. The results of this study contribute greatly to the growing body of knowledge for the eventual implementation of miRNAs for body fluid identification.

#### microRNAs, Body Fluid Identification, Next Generation Sequencing



### H26 A Forensic Volatolomic Approach: A Step Forward in the Characterization of Wound Pathogens

Shawna F. Gallegos\*, Lubbock, TX 79410; Paola A. Prada, PhD, Texas Tech University, Lubbock, TX 79416; Kendra P. Rumbaugh, PhD, Texas Tech University Health Sciences Center, Lubbock, TX 79430

**Learning Overview:** After attending this presentation, attendees will better understand the Volatile Organic Compounds (VOCs) emitted by chronic wound pathogens, both *in vitro* and *in vivo*.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an enhanced understanding of VOCs emitted by wound pathogens and how they can be analyzed to better understand the etiological causes of wound infections. Enhanced and early diagnosis of organisms colonizing a wound are critical to effective treatment and prevention of chronicity. Diagnosis by detecting and identifying VOCs could provide physicians with more rapid and sensitive knowledge of the organisms present in the wound and therefore expedite treatment to prevent complications.

With ongoing conflicts engaging military personnel, there has been a substantial increase in combat-related injury with high rates of morbidity and mortality. Chronic wounds have considerable effects on the patient, both physically and psychologically, as treatment is painful, the wound is slow to heal, and often leads to amputation to prevent sepsis and death. Treatment costs are also substantial, accumulating upward of \$3 billion annually. The persistence of the chronic wound and the related costs is contributed to by microbes that colonize the wound, many of which are highly resistant to antibiotics, creating additional challenges to treatment. These microbes often form biofilms—bacteria encased in their own Extracellular Polymeric Substances (EPS), which compound the healing problem. Biofilms, especially those in the wound environment, are generally polymicrobial with relationships that vary from symbiotic to highly competitive. Diagnosis relies upon standard culture methods to identify the organisms present in the wound. However, some microbes are difficult to culture or may not be present at quantifiable levels, posing additional challenges to effective treatment.

Individual species of microbes have specific molecular makeups, including specific VOCs that are also thought to be unique to each organism. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are two of the most common chronic wound pathogens and are highly multidrug resistant. Utilizing Solid-Phase Microextraction (SPME) and Gas Chromatography/Mass Spectrometry (GC/MS), this study used an *in vivo* model to compare VOCs present in the chronic wound environment to those present *in vitro*. While the number of identified volatile compounds were reduced *in vivo*, these compounds were still comparable to the *in vitro* baseline. Interestingly, in the dual species infection, the dominant compounds belonged to *P. aeruginosa* with lower levels to *S. aureus*. While relationships between organisms in a chronic wound vary from symbiotic to highly competitive, the detected volatile patterns indicate that even at low levels, pathogen VOC profiles vary between species, both qualitatively and semi-quantitatively. This method could lead to the identification of bacterial species present in acute and chronic wound infections without the time needed for standard cultures, as well as overcome limitations with regard to complex and difficult-to-culture organisms.

#### Volatolomics, Solid-Phase Microextraction (SPME), Pathogens



**H27 WITHDRAWN**



## H28 Evaluating Bioinformatic Pipeline Performance for Forensic Microbiome Analysis

Sierra Kaszubinski, BS\*, Michigan State University, East Lansing, MI 48824; Jennifer L. Pechal, PhD, Michigan State University, East Lansing, MI 48824; Heather R. Jordan, PhD, Mississippi State University, Mississippi, MS 39762; Carl J. Schmidt, MD, Wayne County Medical Examiner's Office, University of Michigan, Detroit, MI 48207; M. Eric Benbow, PhD, Michigan State University, East Lansing, MI 48824

**Learning Overview:** After attending this presentation, attendees will understand how available bioinformatic pipelines are applied to forensic microbiology and postmortem microbiome research. Attendees will see that downstream output of multiple pipelines differs based on statistical evaluation, including taxon abundance, diversity metrics, and machine learning model building.

**Impact on the Forensic Science Community:** This presentation will impact the forensic scientific community by informing bioinformatic analysis for further forensic microbiology research and future casework.

While recent research suggests excellent potential for microbial community use in forensics, additional foundational work is needed before forensic microbiology can be applied in the criminal justice system. Downstream bioinformatic analysis needs to be optimized of forensic microbiological data created by high-throughput sequencing platforms in research and case work.<sup>1</sup> To accomplish this goal, a better understanding of performance among bioinformatic pipelines is needed to reveal potentially significant differences in downstream analysis and data interpretation. The ultimate outcome of such evaluation will be the identification of analytical pipelines optimized for use in forensic microbiology.

One aspect of forensic microbiology is examining the human microbiome, which consists of 10-100 trillion microbial cells per person.<sup>2</sup> The composition of these microbial communities can be specific to groups of living individuals and varies by area of the body (e.g., gut vs. skin).<sup>2</sup> Current research on forensic microbiology includes human microbial fingerprinting and estimating time since death using the postmortem microbiome.<sup>3</sup> Advances in sequencing technology have made studying microbes at the genomic level much more affordable. Large-scale microbial identification can be accomplished using 16S rRNA gene sequences obtained from targeted amplicon high-throughput sequencing. These microbial taxon identifications provide a microbiome profile that has potential use in forensics.

Despite increasing research in the use of microbiomes in forensics, there is still a limited understanding of the human postmortem microbiome beyond studies that take place in controlled anthropological facilities.<sup>4</sup> One of the first studies to investigate large-scale ( $n=188$  cases) postmortem microbial changes using high-throughput sequencing and statistical analyses during decomposition revealed dynamic changes for the postmortem microbiome.<sup>5</sup> This research was the largest dataset to provide empirical evidence that microbial communities of the eyes, ears, nose, mouth, and rectum could predict time since death during routine death investigation using high-throughput technology and *in silico* computational tools.<sup>5</sup>

*In silico* tools are needed to analyze forensic microbiology data. For bioinformatic analysis, raw data files undergo a series of transformations using executable command line software known as pipelines.<sup>6</sup> Pipelines most commonly cited are QIIME, mothur, and MG-RAST.<sup>7</sup> Current literature is not sufficient to justify which pipeline would be most useful in analyzing forensic microbial data. Previous studies comparing pipelines either used simulated data, small sample sizes ( $n<40$ ) composed of the same sample type (i.e., human gut microbial data), or *in silico* data.<sup>7-12</sup> Studies analyzing samples of limited sample type and number do not extrapolate to forensic microbiology studies, which often included swabs from multiple body sites.<sup>4,5,13</sup>

Two anatomic areas, the mouth and rectum, were selected from the large and variable dataset from Pechal et al. to quantitatively evaluate each pipeline.<sup>5</sup> Random subsamples were made from the dataset to include 30 cases, 60 cases, 120 cases, and 188 cases. Microbial sequence reads were analyzed with QIIME2 (2017.8), mothur (v.1.39.5), and MG-RAST (4.0.2). The reference database (SILVA) and operational taxonomic unit generation (*de novo*) were controlled. Phylum and family level taxon were used for comparison and revealed a significant difference ( $p < 0.01$ ) in the number of unclassified reads between pipelines. MG-RAST also had fewer taxa present at 1% relative abundance than mothur or QIIME2. The number and identity of taxa shared between anatomic areas, or core taxa, was distinct among pipelines. Alpha diversity metrics, including Shannon and Inverse Simpson diversity indices, were compared among the pipeline outputs. Alpha diversity metrics were significantly different ( $p < 0.01$ ) between MG-RAST and the other pipelines. The pipelines were also compared using random forest, a machine learning algorithm. While predicting anatomic area, the error rates for the model were relatively similar, predicting the correct anatomic area about 95% of the time. Yet, important predictor taxa differed among pipelines. Overall, mothur and QIIME2 had similar results, while MG-RAST was distinct. QIIME2 and mothur can be systematically used for further forensic microbiome analysis.

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### Forensic Microbiology, Postmortem Microbiome, Bioinformatics



### H29 Investigating Commercially Available MicroRNA Extraction Kits for Use With Forensically Relevant Body Fluids

*Autumn T. Muise\**, West Haven, CT 06516; *Karly L. Johannsen*, University of New Haven, West Haven, CT 06516; *Claire Glynn, PhD*, Forensic Science Department, West Haven, CT 06516

**Learning Overview:** The goal of this presentation is to inform attendees of a variety of commercially available microRNA (miRNA) extraction kits and their use with forensically relevant body fluids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the various methods available for miRNA extraction, a variety of methods for quantifying miRNA extracts, and the optimal method for use with forensically relevant body fluids from those tested in this study.

While current research is highlighting the potential of miRNAs for body fluid identification, little research has been performed to investigate the best method for extracting the miRNA content from forensically relevant body fluids. There are more than a dozen commercially available miRNA/RNA extraction kits, with new kits regularly being released onto the market. However, all miRNA extraction kits currently available have been designed for use with pristine clinical laboratory samples, such as cell cultures, primary tissues, plasma/serum, etc. A kit designed specifically for use with forensic samples—venous blood, semen, saliva, menstrual blood, and vaginal material—is not yet commercially available. Therefore, an investigation of some of the currently available, and widely reported, miRNA extraction kits is warranted for use with forensically relevant body fluids. The goal of this research was to select four commercially available miRNA extraction kits, and to assess their ability to extract the miRNA content from forensically relevant body fluids in sufficient quantity and quality for downstream analyses.

Following Institutional Review Board (IRB) approval, body fluids were collected from volunteers with written informed consent. Venous blood was collected by a licensed phlebotomist into EDTA vials. Semen and saliva were collected into sterile conical tubes. Menstrual blood and vaginal material were collected using sterile cotton swabs. All samples were stored at -20°C until extractions were performed.

The miRNeasy Mini Kit (Qiagen®), PureLink® miRNA Isolation Kit (Invitrogen™), magMAX™ mirVana™ Total RNA Isolation Kit (Applied Biosystems®) and High Pure miRNA Isolation Kit (Roche) were the chosen kits in this study. Each miRNA isolation was performed according to the manufacturer's protocol. Following miRNA isolation, the extracts were stored at -20°C until quantitation was performed. The extracts were quantified using the NanoDrop™ One UV/Vis spectrophotometer (Thermo Scientific™), Qubit® 3.0 fluorometer (Invitrogen™), with the RNA HS assay kit, and the Agilent® Bioanalyzer 2100 (Applied Biosystems®) with the small RNA chips. As each kit has a different final elution volume, all results were converted from ng/μL to total RNA (ng) obtained.

Quantifiable amounts of miRNA were collected from all samples. The results generated using the NanoDrop™ One UV/Vis Spectrophotometer showed the miRNeasy kit to be the optimal kit in the majority of the bodily fluids, with yields ranging from 2,844-6,279ng total, except for vaginal material, in which magMAX™ mirVana™ Total RNA isolation Kit yielded higher results. The results were verified using the Qubit® 3.0 Fluorometer. This quantification method further confirmed that the miRNeasy was the superior kit; however, the Qubit® showed that the High Pure Isolation Kit was preferred for the extraction of vaginal material. Agilent's® Bioanalyzer 2100 quantified the miRNA concentration in extracts that held promising results from the Qubit®. Qiagen's® miRNeasy Mini Kit's miRNA concentration values were present for all five of the bodily fluids. miRNeasy's miRNA concentration ranged from 487-12,749ng total. The use of three separate quantification methods did not consistently give the same amounts.

Qiagen's® miRNeasy Mini Kit was overall the optimal kit in extracting miRNA from forensically relevant bodily fluids, with the exception of High Pure miRNA Isolation Kit, yielding higher results in the extraction of vaginal material. Although the miRNeasy is slightly more expensive than the High Pure, the quantity of miRNA eluted is greater, thus offsetting the costs.

These results in the extraction of miRNA using commercially available kits were successful and will aid the forensic science community in future casework.

#### MicroRNA, Extraction, Body Fluids



### H30 Postmortem Submersion Interval (PMSI) Estimation From the Microbiome of Bone in a Freshwater Lake Across 4,750 Accumulated Degree Days (ADD)

Claire M. Cartozzo, MS\*, Virginia Commonwealth University, Richmond, VA 23220; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA 23284; Tal Simmons, PhD, Virginia Commonwealth University, Richmond, VA 23284

**Learning Overview:** After attending this presentation, attendees will understand how changes in microbial communities across ADD can be used to predict the long-term PMSI for skeletal remains recovered from a freshwater lake.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing proof of concept concerning a novel area of research, the use of microbial succession on skeletal remains submerged in a freshwater lake to predict PMSI.

Aquatic decomposition, the process of remains progressing from submerged fresh remains, to remains floating in a stage of decay, and finally sunken remains, varies due to water temperature, salinity, depth, current, scavengers, three-dimensional movement, and other extrinsic factors. Being able to estimate time since death, or in the case of water-related incidents the postmortem submersion interval (PMSI), is pertinent to Medicolegal Death Investigations, as it assists in identifying remains, corroborating eye witness statements, and narrowing suspect pools. Because microorganisms are present throughout decomposition, researchers proposed that their succession pattern, like insects, could be used to estimate PMI.<sup>1</sup> Recent studies by Dickson et al. and Benbow et al. have demonstrated that bacterial communities in aquatic environments can be a useful tool for estimating PMSI due to advancements in metagenomic approaches (e.g., next-generation sequencing and pipeline analysis software).<sup>2,3</sup> Unfortunately, these studies were performed with small sample sizes ( $n \leq 3$ ), a short sampling period (21–42 days), repeated sampling and disturbance of the same remains. In addition, the focus of these studies was not on microbial community structure of skeletal remains, which is addressed by this research.

In this study, fresh pig (*Sus scrofa*) bones ( $N=100$  rib and  $N=100$  scapula samples) were obtained from a butcher. From November 2016 to June 2018, bones were placed in cages attached to a flotation device and submerged in Henley's Lake (White Hall, VA 38° 05' 11.7"N, 78° 41' 02.8" W). Water temperature was recorded hourly using waterproof loggers. Every 250 ADD, five scapulae, five ribs, and 500ml of water were collected and stored at either -80°C or 4°C until processed. Bone samples were cut and ground into a powder using liquid nitrogen in a mortar and pestle; meanwhile, water samples were filtered on 0.22µm filters. DNA was extracted and purified using ChargeSwitch® gDNA Plant Kit and DNeasy PowerClean® Pro Cleanup Kit, if necessary. Following parameters set forth by Kozich et al., extracted samples were used to carry out sequencing-by-synthesis of microbial 16S rDNA variable region 4 using Illumina's® MiSeq® 2X300 paired-end sequencing.<sup>4</sup> The resulting data were analyzed via MiSeq® SOP mothur version 1.36.1.<sup>5</sup>

According to preliminary data encompassing baseline through collection 5 (0–1,250 ADD), Analysis of Molecular Variance (AMOVA) indicate a significant difference in the bacterial structure between sample types, specifically rib-scapula, ( $p < 0.0002$ ), rib-water ( $p < 0.0002$ ), and scapula-water ( $p < 0.0002$ ). For each sample type, phylum and family level changes were observed across ADD. In addition, rib ( $R^2=0.48$ ) and scapula ( $R^2=0.64$ ) samples demonstrated a positive relationship between Shannon species diversity and logADD, whereas the water samples showed a negative relationship ( $R^2=0.48$ ). Similar differences and trends are expected with the remaining 13 collections.

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#### Postmortem Submersion Interval, Freshwater Bone, 16S rRNA Gene



### H31 The Mouse Thanatobiome and Postmortem Interval (PMI) Estimation

Molly B. Still\*, Emporia, KS 66801; Scott S. Crupper, PhD, Emporia State University, Emporia, KS 66801

**Learning Overview:** The goal of this presentation is to offer an alternative method for determining the PMI using the thanatobiome as examining changes in microbial succession patterns may satisfy this objective.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping to address current problems in PMI estimation and showing how thanatobiome data may offer an alternative. Although the thanatobiome is a relatively new field of study, data acquired may offer an alternative to PMI determination.

Determining an accurate PMI in a death investigation is a critical piece of information needed to gain a thorough understanding of the circumstances surrounding a death. While different methods are available to forensic investigators, each has its own set of limitations that decrease its accuracy. Microbes are ubiquitous and play a significant role in decomposition. The thanatobiome is a term used to describe the microbes that colonize internal organs postmortem. Since there is dramatic shifting in microbial communities within a deceased host, these unique microbial signatures may be utilized by forensic investigators to establish a PMI.

Previous PMI studies have employed animal models, such as swine and rodents, since it is easier to control the PMI and obtain multiple replicates as compared to human cadavers. To gain a better understanding into the use of the thanatobiome for PMI studies, this study utilized a mouse model system to examine microbial succession patterns in the liver postmortem. Mice were sacrificed by CO<sub>2</sub> asphyxiation and placed at room temperature in sealed containers that allowed air flow. In total, mice were separated into four groups: a control group (0-day postmortem), and 7-, 15-, and 21-day postmortem groups. At the indicated PMI, livers were removed and stored at -80 C. Total DNA was isolated from each liver sample using a Zymo Research Fungal/Bacterial DNA MiniPrep kit according to the manufacturer's recommendations. Subsequently, the V3 hypervariable region of the 16S rRNA gene was amplified and sequenced on an Illumina® MiSeq® platform. The results indicate *Clostridium* species dominated in all three PMI groups, whereas *Lactobacillus* species accounted for only a small proportion of the total thanatobiome. However, in two of the 21-day PMI mice where the total percentage of *Clostridium* species was significantly lower, *Lactobacillus* species accounted for most of the genera identified. Noteworthy is the fact that putrefaction in the 21-day postmortem mice had progressed to a level that significantly liquefied and made the liver difficult to recognize and harvest. Furthermore, the Shannon Species Diversity Index, which measures species richness, significantly increased from approximately .021 in the control groups to 0.78–2.4 in the 7- to 21-day PMI groups, illustrating that decomposition is a process mediated by a wide variety of microbes. In total, results obtained agree with previous human cadaver thanatobiome studies that have noted a “*Clostridium* Postmortem Effect.”

#### Thanatobiome, PMI, Clostridium



## H32 Postmortem Hair Microbiome and Its Forensic Applications

Denise Wohlfahrt, BS\*, Virginia Commonwealth University, Richmond, VA 23284; Kailey Babcock\*, Richmond, VA 23220; Courtney Hutchens, BS, Virginia Commonwealth University, Richmond, VA 23284; Vadim Mesli, MD, Institut Médico Légal/Forensic Institute, Nord 59037, FRANCE; Erwan Le Garff, MD, Institut Médico Légal/Forensic Institute, Nord 59037, FRANCE; Valéry C. Hedouin, MD, PhD, Hauts De France 59037, FRANCE; Xavier Demondion, MD, PhD, Laboratoire D'anatomie, Nord 59000, FRANCE; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA 23284

**Learning Overview:** After attending this presentation, attendees will better understand bacteria associated with human hair samples collected after death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping forensic scientists find new ways for analysis of hair evidence during death investigation.

Human hair is often encountered at crime scenes. However, those hairs most often found don't often contain nuclear DNA needed for the current human DNA workflow. This survey of the microbiome associated with human hair can aid researchers in determining whether hair can be useful in Postmortem Interval (PMI) estimation as well as in determining whether the area from which the hair is collected is important. This analysis was performed as part of an ongoing investigation into the microbiome of hair samples. Previous research has shown that in living humans, bacteria associated with scalp hair are significantly different from those bacteria associated with pubic hair in both sexes. We also know that bacteria associated with pubic hair of males are significantly different than those bacteria associated with pubic hair of females. One question that remains unanswered is how hair microbiome is affected after death, and whether the scalp hair microbiome changes depending on the area of the scalp it is collected from. To understand this, scalp and pubic hair samples were collected from ten human cadavers from the United States (Office of the Chief Medical Examiner, Richmond, VA) and from 25 human cadavers from Europe (Medicolegal Institute of Paris, France). For the European population, scalp hair samples were collected from frontal, occipital, left and right parietal, and vertex regions of the scalp, whereas for the American population, scalp hair samples were collected from a single location. Pubic hair samples were collected from a single location in the pubic area in both populations. All samples were stored at -80°C. DNA was extracted from approximately one inch of finely cut hair samples using the Tridico et al. method with the addition of zirconium bead beating.<sup>1</sup> Samples were then amplified using dual-index 16SrDNA MiSeq<sup>®</sup> sequencing using the protocol as described by Kozich et al.<sup>2</sup> Sequences were then analyzed using mothur version 1.39.4, and statistical analysis was performed using R version 3.4.0.<sup>3,4</sup>

Preliminary results indicate that the bacterial structures associated with scalp hair are significantly different from those associated with pubic samples. The majority of bacteria found in scalp and pubic hair samples belong to the phyla Cyanobacteria, Proteobacteria, Spirochaetes, and BRC1, varying only in their relative abundances. When comparing the genera, pubic hair samples show much higher relative abundance of *Staphylococcus*, where scalp hair showed a higher relative abundance of *Selenomonas*, *Vagococcus*, and *Streptococcus*. However, initial tests did not indicate significant variation between male and female pubic or scalp hair samples. As the majority of samples were obtained from Caucasian donors, no determination was made on whether race impacted the microbiome of hair samples.

In conclusion, this ongoing study provides information on the bacterial communities associated with hair samples collected from human remains. This information will help provide better understanding of the usefulness and utility of hair associated microbial communities in forensic investigations.

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### Hair Microbiome, Necrobiome, 16S rDNA



### H33 Forensic Microbiology: An Analysis of a Series of Cases

*Elvira Ventura Spagnolo, MD\*, University of Palermo, Palermo 90127, ITALY; Cristina Mondello, MD\*, Department BIOMORF, University of Messina, Messina 98123, ITALY; Salvatore Rocuzzo, Caltagirone 95041, ITALY; Francesco Daleo, MD, Department of Clinical and Experimental Medicine, Messina, ITALY; Stefania Zerbo, MD, Via Del Vespro, 127, Palermo 90100, ITALY; Antonina Argo, Palermo, Europe 90100, ITALY*

**Learning Overview:** After attending this presentation, attendees will be able to evaluate the importance of microbiology for forensic investigations and the importance in creating a standardized protocol to improve its practical application.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting how forensic microbiology could be a useful tool to determine the cause and manner of death, especially in difficult forensic cases such as sudden unexpected death or death due to nosocomial infections.

Postmortem microbiology is a relatively novel research field with great potential concerning forensic field applications. Nevertheless, an effort is needed to solve actual controversies on the correct interpretation and statistical significance of a postmortem cultural result. To this end, a multidisciplinary approach would be useful. A large part of the issue is relative to the lack of standard procedures for the collection and analysis of samples. Relying on the studies so far produced, a correct approach requires: correct storage of the body at 4°C to prevent bacterial translocation; collection of samples within 24h and 48h from death prior to evisceration of the body; the use of appropriate collection media; sterilization of the surfaces of the body sites chosen using, for example, a hot spatula or iron; the use of sterile tools (e.g., sterile needles); and immediate transfer of the samples collected to the microbiology laboratory.

According to the Human Microbiome Project (HMP), a healthy human body contains ten times more microbes than human cells. Microbial communities colonize different organs of the body, playing fundamental roles both in human health and disease. Despite the vast scientific knowledge on the role of microbial communities in a living body, little is known at present about microbial changes occurring after death.

The study investigated 35 autopsies performed by the standard techniques in which cardiac and femoral blood, pericardial fluid, pleural fluid, urine, pieces of lung, spleen, and liver were obtained. The sampling was conducted according to the rules of asepsis indicated by Riedel et al. and according to the procedures issued by the European Congress of Clinical Microbiology & Infectious Diseases (ESCMID) Study Group for Forensic and Postmortem Microbiology (ESGFOR) study group to exclude contamination or postmortal translocation of microbes.<sup>1</sup>

The microbiological results showed positivity for both ubiquitous and multi drug resistance microorganisms (such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*). These results provided data to confirm infections diagnosed during care assistance and, in particular, to assess the responsible microbes in cases without pre-mortem microbiological analysis. It was also observed that in cases of health care-related infections, the microorganisms presence were found in all the examined samples (biological fluids and body tissues), supporting the clinical diagnosis of a severe septic state. Obviously, the interpretation of these results must be integrated by circumstantial, clinical, autopsy, and histological findings. Moreover, it is important to critically analyze the microbiological results considering possible bacterial contamination and a general effort is thus needed to obtain standardized protocols to demonstrate the real potential of this recent research field.

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#### Postmortem Microbiology, Sepsis, Forensic Analysis



### H34 Calliphoridae of Forensic Importance of Campeche, Mexico

*Carolina Núñez-Vázquez, PhD\*, Carolina Núñez Vazquez, Merida, Yucatán 97168, MEXICO*

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**Learning Overview:** The goal of this presentation is to help attendees better understand the diversity of species of Calliphorids of forensic importance in Campeche, Mexico.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data not previously generated in Mexico.

The objective of this study is to know the diversity of species of Calliphorids that can be found in the locality of Campeche, Mexico, with the purpose of this information forming part of a national database as part of a larger project of georeferencing and seasonality of insects of forensic importance in Mexico. This database will serve as a reference for forensic investigators, experts, scientific researchers, or students since, in Mexico, forensic entomology is little known and therefore little applied in the legal system. One of the basic needs for the development and implementation of forensic entomology in the country is that the presence, distribution, and seasonality of the insect species that may be involved in a forensic case must be known. Knowing the presence or absence of these insects at different seasons of the year can help to establish the season in which the death occurred; knowing the distribution of insects can help in investigations that involve relocation of corpses, origin of some drugs, location of a suspect in the scene, and locating where a vehicle has traveled, among others.

The site that was chosen to conduct the insect collection is the town of Pucnachen in the state of Campeche, located in southeast Mexico. This region has an altitude of 5 meters above sea level, the soils are rocky with little organic matter, has a semi-flat surface, and few elevations that reach 2.5 meters in height. Its climate is mostly warm-subhumid and rainy during the summer. The average annual temperature is 27.1°C and has vegetation belonging to the low deciduous forest.

The insect collection took place in March and April. Six traps baited with pork and alcohol as a method of preservation of insects were used. The traps were placed on trees at a height of 2 meters to prevent other scavengers, such as dogs, from destroying them and were separated at a distance of 30 meters. The traps were monitored every week for eight weeks. All collected insects were transferred to the laboratory for identification and preservation. Currently, this work is in development, so results are preliminary, and the collection work will be replicated in other seasons. The information generated will be part of the database for future reference.

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#### **Forensic Entomology, Pucnachen, Calliphoridae**



### H35 Development of the Discrimination Procedures Between Nasal Secretion and Saliva by Real-Time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) and Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

Tomoko Akutsu, PhD\*, National Research Institute of Police Science, Kashiwa, Chiba 277-0882, JAPAN; Ken Watanabe, National Research Institute of Police Science, Kashiwa, Chiba 277-0882, JAPAN

**Learning Overview:** After attending this presentation, attendees will understand the applicability of rRT-PCR and ELISA procedures on nasal secretion- and saliva-characteristic markers for the discrimination of these body fluids through analysis of specificities and sensitivities of these procedures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing reliable information on the source of human DNA through the identification of body fluids, which are often left at crime scenes but are difficult to distinguish from each other.

In forensic casework, nasal secretions could be a good source of DNA for the identification of individuals. There are, however, few procedures to identify nasal secretions. Further, saliva that is often left at crime scenes could prove sexual assault. However, presumptive and confirmative tests for saliva on the basis of  $\alpha$ -amylase can cross-react with other body fluids, such as nasal secretions. In previous studies on messenger RNA (mRNA) - based identification of body fluids, some nasal secretion- and/or saliva-characteristic genes have been reported; however, end point detection of these genes may be difficult to discriminate from each other because of their insufficient specificity and detectability. In addition, there are no reports of protein marker(s) for the identification of nasal secretions. Furthermore, ELISA detection of Statherin (STATH), commonly used as a saliva marker, cross-reacted with nasal secretions.

Therefore, the goal of this study was to develop more specific procedure(s) for discrimination between nasal secretions and saliva for forensic purposes. Candidate molecules for the identification of nasal secretion (bactericidal permeability increasing protein fold containing family A member 1 ((BPIFA1), STATH) and saliva (STATH, histatin 3 (HTN3)), and proline-rich protein HaeIII subfamily 2 (PRH2)) were selected, and RT-PCR and indirect ELISA procedures were developed to determine these mRNA and protein expression levels quantitatively.

Expression levels of candidate genes were determined in various body fluids and discrimination criteria for nasal secretions and saliva were determined based on quantitative results of multiple markers. In addition, a flowchart to discriminate among nasal secretions, saliva, and other body fluids was proposed and evaluated on various forensic samples. Similarly, the specificity and sensitivity of ELISA detection of candidate proteins were determined using various body fluids, and a positive threshold value was set for each candidate marker. Then, applicability of ELISA procedures to the forensic casework was investigated using simulated casework samples.

As a result of real-time RT-PCR analysis, BPIFA1 was highly but incompletely expressed in nasal secretions and expressed in semen and vaginal fluids in trace levels. STATH was expressed in almost all the nasal secretion and saliva samples but not detected in other body fluids analyzed in this study. HTN3 was specifically expressed in saliva samples as reported previously. Unexpectedly, only a few saliva samples showed positive results in RT-PCR analysis for PRH2 though it was reported as a specific protein marker for saliva. Using determined discrimination criteria and the proposed flowchart, nasal secretions and saliva were successfully discriminated among various body fluids analyzed in this study.

The results of ELISA analysis showed that BPIFA1 was specific to nasal secretions. Besides, STATH was detected in both nasal secretion and saliva samples in accordance with previous reports and results of gene expression analysis. PRH2 was specifically detected in most saliva samples despite low gene expression levels in saliva. Development of ELISA detection of HTN3 was not achieved because suitable antibodies were not found in this study.

In conclusion, for the discrimination of nasal secretions and saliva among various body fluids, BPIFA1, STATH, and HTN3 could be effective markers for RT-PCR. On the other hand, BPIFA1, STATH, and PRH2 could be useful as protein markers for ELISA. Suitable procedures could be selected depending on the sample condition and sample type because environmental tolerance between mRNA and protein was thought to be different.

#### Nasal Secretion, Saliva, Body Fluid Identification



### H36 The Estimation of Postmortem Submersion Interval (PMSI): Are Nails' Ultrastructure of Any Help?

*Silvia D. Visona, MD, University of Pavia, Pavia 21100, ITALY; Ilenia G. Tredici, PhD, CISRiC, University of Pavia, Pavia I27100, ITALY; Elena Mercuri\*, Pavia 27100, ITALY; Chiara Siodambro\*, Pavia 27100, ITALY; Matteo Moretti, MD, University of Pavia, Pavia 27100, ITALY; Luisa Andrello, MD, Bellinzona, Canton Ticino 6500, SWITZERLAND; Gulnaz T. Javan, PhD, Alabama State University, Montgomery, AL 36104; Giovanni Fassina, Pavia, Lombardia 27100, ITALY; Antonio M.M. Osculati, MD, University of Pavia, Pavia 27100, ITALY*

**Learning Overview:** The goal of this presentation is to provide new tools for the estimation of the PMSI in bodies recovered in freshwater.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing a scarcely investigated issue that can provide helpful information regarding the circumstances and time of the death.

Forensic pathologists face numerous challenges when dealing with corpses submerged for several days. In such cases, the pathologist encounters many difficulties in determining the time since death, as the submersion deeply alters the postmortem phenomena. Therefore, it is of great interest to be able to assess how long the corpse remained in water, regardless of the PMI. This data can be helpful to reconstruct the circumstances of the death. Several approaches have so far been proposed for the PMSI estimation. Methods involving the growth of biofilms, such as algal, bacterial, fungal, and protozoan species, have been reported in the literature with encouraging results. Another tested method used to estimate the PMSI involves measuring the degree of decomposition of the recovered human remains. In the present study, a novel approach was tested, consisting of examining the microscopic structure and the ultrastructure of the nail plate, experimentally submersed in freshwater. The structure of the nail unit has been well described in literature. The nail plate is the completely keratinized part of the nail unit and it is made up of three different layers that are well-bonded together. Nail matrix produces the nail plate that includes an average of 196 cell layers that are distributed among three tightly bound layers. Given its very low permeability to water, due essentially to the chemical composition of the nail containing residual lipids, the penetration of water into the nail plate is likely to be slow. These characteristics lead to the hypothesis that the changes in the nail structure due to submersion in water develop gradually, going through different stages.

To observe these modifications, the nail plate of the hallux was removed from five cadavers during forensic autopsies. Each nail was experimentally kept in freshwater at room temperature for defined time intervals: 3, 6, 9, 12, and 24 days. As a negative control, a dry hallux nail, removed as well from a cadaver, was used. As positive controls, the hallux nails of two cadavers pulled from the water, respectively, after 9 and 24 days, were examined. Each nail was formalin fixed for 24 hours, then dehydrated in a graded series of ethanols (60%, 80%, 95%, 100%). Then, after drying, each nail was longitudinally divided into two parts, to expose the internal structure of the nail, rather than the external surface. Each sample, previously carbon-coated using Cressington carbon coater 208c, was examined with a Scanning Electron Microscopy (SEM) Tescan® Mira3XMU, operated at 20kV.

The results showed that a relevant different pattern can be observed for each experimental submersion interval. High-power views indicate that the nails not submerged or submerged for the shortest interval (3 days) show a compact structure, with regularly aligned keratin fibers. After 6 days, some cracks are observed, and they appear considerably more extended after 9 days; after 12 days of submersion, the nail plate is characterized by the apposition of exogenous material, such as diatoms. After 24 days of submersion, the structure is totally altered, with extensive discontinuities and it is no longer possible to distinguish the different layers of keratin, which appear to be compacted and amorphous.

Such results suggest the usefulness of the ultrastructural observation of the nails using the SEM in the evaluation of the PMSI in bodies recovered in freshwater. Indeed, the observed changes seem to follow a gradual process. The characteristics pointed out in this presentation, even though they must be validated and confirmed by more research, appear promising and may be extremely useful in the forensic practice.

#### Scanning Electron Microscopy, Nail, Postmortem Submersion Interval



### H37 Intentional Body Dismemberment: A Difficult Path for the Forensic Pathologist in the Search for Truth

Aniello Maiese, *Sapienza University of Rome, Rome 00185, ITALY*; Alessandra De Matteis, *University Sapienza of Roma, Rome 00100, ITALY*; Matteo Scopetti, MD\*, *Sapienza University of Rome, Rome, ITALY*; Alessandro Santurro, MD, *Sapienza, University of Rome, Rome 00161, ITALY*; Vittorio Gatto, MD, *Sapienza University of Rome, Roma 00185, ITALY*; Mariantonia Di Sanzo, MD, *Rome, ITALY*; Luigi Cipolloni, MD, PhD, *Rome 00161, ITALY*; Massimiliano dell'Aquila, MD, *Sapienza University, Department of Anatomy, Rome, Lazio 00169, ITALY*

**Learning Overview:** After attending this presentation, attendees will better understand the importance of a systematic approach to intentional body dismemberment cases, based on radiological, autoptical, genetic, histological, immunohistochemical, and toxicological investigations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an overview of the most recent cases of dismemberment that occurred in Italy in the past eight years.

The term “dismemberment” is used to indicate the detachment of the limbs and/or the head from the trunk at the level of the respective joints, or the subdivision of the thorax, the abdomen or the limbs into the respective segments. Three cases of intentional body dismemberment by another individual have been analyzed at the Morgue – Umberto I General Hospital/Sapienza University of Rome to determine the identity of the victim, the causes of death, and the wound vital reaction. The identity of the victim had been ascertained during the crime scene investigation, conducted by data and photographic recovery, and subsequently confirmed by the genetic exam, which also allowed confirmation of the assignment of all the body parts to the same subject.

In one case, dismemberment of the lower limbs has been performed. In the second, both the upper limbs and the lower limbs had been detached as well as an evisceration of the thoracic and abdominal organs. In the last case, the corpse had been dismembered meticulously, so much so that it was difficult to even identify the various anatomical segments during the crime scene investigation.

In all cases, a preliminary radiological study by a total-body Postmortem Computed Tomography (PMCT) was conducted and subsequently a complete autopsy exam was performed; on the tissue samples taken at autopsy, a classic histological exam with hematoxylin/eosin and an immunohistochemical study with IL-15, CD-15, and triptase were performed in order to evaluate the wound vitality at the incised planes. Finally, with gas chromatography, toxicological tests were conducted on the biological fluids (central and peripheral blood, urine, and vitreous humor) to identify exogenous substances.

From these investigations, it was possible to identify the cause of death: in two cases, it was by violent mechanical asphyxiation perpetrated by strangulation, while in the other, it was attributed to a sharp-force injury penetrating the abdomen. Histological and immunohistochemical results have shown that the depletion had occurred postmortem in all three cases; the toxicological investigations, on the other hand, yielded positive results for exogenous substances (heroin) in one case, but not in such a concentration as to determine the death.

In all the examined cases, the dismemberment followed the murder of the victim. This practice is in fact usually performed to make the body more easily concealable; in all three cases, the perpetrator of the crime had hidden the remains of the body in plastic bags, suitcases, or similar containers to hide them. Another detail common to all cases is the accidental finding of the remains by pedestrians who passed through the area, showing that the meticulousness performed in fragmenting the victim was not applied to the concealment of the bodies.

#### Dismemberment, Forensic Pathology, Homicide



### H38 Buried Secrets: A Multidisciplinary Approach to Solving a Half-Century-Old Homicide

*Elizabeth M. Sepulveda, BA\*, Islip, NY 11751; Ruth E. Kohlmeier, MD, Suffolk County Office of the Medical Examiner, Hauppauge, NY 11788-0099*

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**Learning Overview:** After attending this presentation, attendees will understand how law enforcement and the different forensic specialties interact, the importance of teamwork in solving cold cases, and what an impact police investigations and forensic sciences can have on the surviving family members and loved ones of the deceased.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by revealing how a team of investigators and forensic scientists worked together to solve a half-century-old homicide.

In early October 1966, a Long Island woman disappeared, leaving behind her 11-year-old daughter. She was last seen getting into her boyfriend's vehicle and was never seen alive again. In an era of hushed mouths and limited investigative resources, the mystery and scandal surrounding this woman's departure remained unknown until March 2018. It was a reporter with the *Suffolk Times* who took an interest and began to dig deeper, which led to a guilt-ridden widow's confessions being told. Law enforcement agencies, including the New York State Police, the Southold Police Department, and the Suffolk County Police Department investigated further and eventually interviewed the boyfriend's ex-wife. She described to police how her husband had placed this woman in a burlap sack and then buried her in a grave he had dug in the basement of their home in eastern Long Island.

After three attempts at locating this missing woman, in March 2018, the remains were found in the basement of the boyfriend's house approximately six feet below the surface. In addition, two .38 caliber projectiles were also recovered from the grave. The forensic anthropologist stated there was near complete recovery of the skeleton and described the remains as being that of a Caucasian female, 22 to 50 years of age, and approximately 5 feet 6 inches in height. There was no trauma identified. Although there were no antemortem dental records available, the forensic dentist was able to conduct a survey and noted a moderate amount of restoration. The restoration material was then analyzed by a colleague, and it was noted that the material was in use in the 1960s. The morgue supervisor, along with a deputy medical examiner and firearms examiner with the Suffolk County Medical Examiner's office, examined the recovered clothing and noted defects on the front of various articles of clothing that were consistent with gunshot wounds. Finally, the woman was positively identified via DNA using exemplars from the decedent's daughter and brother.

This case exemplified extraordinary teamwork, which led to the discovery, identification, and cause of death of a woman who had been missing since 1966. This case also demonstrates the impact law enforcement investigations and forensic science can have on the surviving family members and loved ones of the deceased.

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#### Homicide, Cold Case, Forensic



### H39 Suicides Among the Young: A Ten-Year (2007–2017) Retrospective Analysis of Suicides Under the Age of 18 in Metro Detroit

Vera Mendes-Kramer, MA\*, White Lake, MI 48383; Lynette Desjarlais, BA\*, Wayne State University, Detroit, MI; LokMan Sung, MD, Detroit, MI 48207; David Moons, MD, PhD, Detroit, MI 48207; Leigh Hlavaty, MD, Wayne County Medical Examiner's Office, Detroit, MI 48207; Avneesh Gupta, MD\*, University of Michigan, Ann Arbor, MI 48109

**Learning Overview:** After attending this presentation, attendees will understand the growing trends of suicide-related deaths within adolescence during a ten-year period in the Wayne County Medical Examiner's Office, and how they correlate with those found at the national level.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing child suicide prevention and the need for increased therapy and follow-up, as evidenced by child suicide data reviewed by the Wayne County Medical Examiner's Office.

The United States has seen suicide rates steadily increase within the past 30 years, with suicide currently being the tenth-leading cause of death nationally and the second cause of death among youths 10-17 years of age.<sup>1,2</sup> To assess Michigan trends in suicidal youth, the Wayne County Medical Examiner's Office conducted a retrospective study of suicides in the Metro Detroit area from 2007 to 2011, and again from 2012 to 2017.

A total of 2,513 suicide cases were reported for all age groups between the years 2007 and 2017, of which 103 suicides fell within the 0-17 age group, comprising 4% of the total number of suicides. The number of suicides in youths, ages 0-17 years, has increased by 39.5% in the last 5 years in comparison with the previous 5 years. The data gathered for the two comparison periods observed the highest numbers of suicide taking place between 16 to 17 years of age and recorded the youngest suicide at the age of 7 years ( $n=1$ ).

In addition to age, data was collected for gender, suicidal method, reported bullying, medical conditions, indication of previous suicide attempts, the type of note left, and toxicology findings for the two five-year periods. The rate of suicide is 3.5 times more prevalent in males than females; 70% of suicides are attributed to males and 30% to females in the overall gender distribution. A comparison between 2007–2011 and 2012–2017 in suicide by gender also attributed a higher number to males in both groups, with a 20% increase in the last five-year period.

The number of suicide deaths by method was compared to national trends and, contrary to national data where firearms hold the place for leading suicide method over suffocation/hanging and drugs for all age groups, at Wayne County, hanging was the chosen method for the 2007–2017 period with 66% of attributed deaths, followed by firearms with 21%, drugs with 7%, multiple injuries with 5%, and drowning with 1%.<sup>3</sup> Though deaths attributed to firearms increased from 16% to 25% in the past five years, the method of hanging, though decreased from 72% in 2007–2011 to 61% in 2012–2017, is still leading the suicide death by method of choice.

Bullying is a multifaceted developmental issue that stems from issues forming or holding relationships that manifest into aggression in peak ages of 9-15 and increase the likelihood of self-inflicted harm in those that participate in or are victims of bullying.<sup>4,5</sup> This study found that the second-largest age distribution occurred in ages 13-15 ( $n=42$ ), which aligns itself with the idea that bullying may predispose children to self-harm.<sup>6</sup>

The number of prior suicide attempts increased from 19% in 2007–2011 to 27% in 2012–2017. Of the 103 suicide cases recorded, 23% ( $n=24$ ) presented with at least one prior attempt, and 23 of the 24 cases reported decedents suffering from depression at the time of demise.

Twenty-five percent of the suicide victims of this study left a suicide note behind, either paper-based, verbal, or in social media. The type of note left changed over the years, particularly the increase in social media notes, from non-existent in 2007–2011 to 8% in 2012–2017.

This study highlights the veer from the national trends in suicide deaths by methods and recognizes new developments that merit mention and for which further research is recommended: the impact bullying has on increasing suicides in youths, the number of prior suicide attempts, and the type of suicide notes left behind.

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#### Suicide, Young, Bullying





## H40 Difficult Suicide Cases: A Case-Based Approach

Wendy M. Gunther, MD\*, Office of the Chief Medical Examiner, Tidewater District, Norfolk, VA 23510-1046; Michael Alan Hays, MD, Office of the Chief Medical Examiner, Tidewater District, Norfolk, VA 23510-1046

**Learning Overview:** The goal of this presentation is to review cases of suicide with conflicting manner-of-death indicators from scene, history, and autopsy, as well as compare case-based methods of differentiating difficult suicides from homicides or accidents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) providing a case-based approach to the analysis of difficult suicides; (2) analyzing conflicting manner-of-death indicators by scene, history, and autopsy findings; and (3) suggesting alternate testing and extended scene investigation for difficult suicides.

Differentiating cases of suicide from homicides or accidents is not always straightforward. Factors that may complicate determination of a suicidal manner include unclear history, scene investigation findings that do not point clearly to suicidal intent, and autopsy findings, including unusual or atypical drugs or injuries.<sup>1</sup> Deliberate self-harm can be difficult to determine in drowning and overdose deaths, which may result in assignment of an undetermined manner; this may contribute to undercounting of suicides in vital data.<sup>2</sup> Unclear history, scene indications, and autopsy findings may contribute to medical examiner/coroner difficulties with families who do not accept the manner of suicide.

Difficult suicide cases are likely to lack a clear history of suicidal ideation, medical records of psychiatric illness/depression, or reported motive for committing suicide. Hanging suicides may create family suspicion depending on their understanding of the degree of suspension required to result in death. A problematic history may include involvement of another person. If this person is a romantic partner whose behavior is deemed suspicious by the family, additional investigation and autopsy maneuvers may be indicated to indict or clear the alleged assailant. Difficulties may be encountered when death occurs during a struggle between two people over a weapon.

This review of difficult suicide cases from the Tidewater, VA, Office of the Chief Medical Examiner (OCME) experience includes cases with unclear histories, complicated scene investigations, and atypical injuries. Difficult history findings in this series include lack of a note or a texted warning, atypical drug abuse in overdose deaths, and behavior before death atypical for suicide, such as celebrating at a bar, going to the beauty parlor, or caring for small children without other arrangements to provide for them. Additional history/scene investigation factors include romantic partners who fled the scene or insisted on hospitalization for emotional distress prior to a police interview.

Difficulties in scene investigation may include decomposition artifacts, premises that are not secure, a body under water, unclear suicide notes or suicide notes that appear to have been written after death, and scenes that have been altered prior to investigation; these may require extended investigation. Complicated scenes reported in this case series include struggle over a gun, allegedly running from the home prior to being found unresponsive in a ditch, discovery of a decomposed missing person in a marshy area, and intraoral gunshot wound while in handcuffs during the process of arrest.

Atypical injuries may present difficulties in determining manner. These include gunshot wounds that are not hard contact, gunshot wounds or sharp force injuries in unusual locations, multiple gunshot wounds or sharp force injuries, and poisoning with unusual drugs. Atypical injuries reported in this case series include a tangential gunshot wound with stippling, sharp force injury to the wrists without tendon or vascular involvement, and drowning with unexplained internal neck injuries.

Ancillary studies that may be useful to clarify cases like these include gunshot residue testing for the decedent and the alleged assailant, blood spatter analysis at the scene, extended toxicologic analysis, and extension of the scene investigation to correlate it with autopsy findings. This series of cases from the Tidewater, VA, experience illustrates an approach to difficult suicides that may be useful to the medical examiner/coroner.

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### Suicide, Atypical Gunshot Wounds, Case-Based Analysis



## H41 A Broad Demographic Analysis of Chronic Ethanol Deaths and Sequelae From Two Different Medical Examiner Offices

Anastasiya Haponyuk, BSc, University of New Mexico School of Medicine, Albuquerque, NM 87122; Sarah Lathrop, DVM, PhD, Albuquerque, NM 87111; Lori A. Proe, DO, Office of the Medical Investigator, Albuquerque, NM 87102; Daniel W. Dye, MD, Jefferson County Coroner/Medical Examiner Office, Birmingham, AL 35233; Matthew D. Cain, MD\*, Office of the Medical Investigator, Albuquerque, NM 87102

**Learning Overview:** After attending this presentation, attendees will understand the demographics of chronic ethanol-related deaths in New Mexico and Jefferson County, AL.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting trends associated with chronic ethanol deaths across racially and ethnically diverse populations.

**Background:** Mortality due to cirrhosis in the United States has increased substantially over the past two decades. Liver disease caused by chronic ethanol abuse has fueled this increase and has disproportionately affected younger people.<sup>1</sup> Ethanol-related mortality, particularly liver disease caused by ethanol, has disproportionately affected American Indian populations.<sup>2</sup>

New Mexico's population is 2,103,586 and is comprised of 8.6% American Indian/Alaska Native, 1.7% Asian/Pacific Islander, 2.2% Black/African American, 48.5% Hispanic, and 39% non-Hispanic White. In the United States as a whole, 0.8% of the population is American Indian/Alaska Native.<sup>3</sup> Ethanol-related mortality rates in New Mexico have historically been highest among American Indians and, while ethanol-related mortality declined among non-Hispanic Whites in New Mexico over recent decades, it has remained high in American Indian populations.<sup>4</sup>

**Purpose:** The intent of this study is to compare ethanol-related deaths in New Mexico to those in Jefferson County, AL, by examining data from the medical examiners' offices in both locations. Jefferson County's population is estimated at 659,197, with 53.2% non-Hispanic Whites, 43.4% Black/African American, 0.3% American Indian/Alaska Native, 3.9% Hispanic, and 1.8% Asian/Pacific Islander.<sup>5</sup> By comparing ethanol-related mortality between these racially diverse populations, this study will identify demographic trends, particularly related to the ages at which people succumb to complications of ethanol abuse, and will also examine autopsy findings indicating an ethanol-related death.

**Methods:** To examine a diverse population, five years of data were retrieved from the New Mexico Office of the Medical Investigator (OMI) and Jefferson County Coroner/Medical Examiner (JCCMO) databases. Cases included natural deaths directly related to chronic ethanol abuse. Analysis of chronic ethanol abuse-related diagnoses used only cases with autopsy verified findings.

Analysis was performed using Statistical Analysis Software (SAS). This study analyzed continuous variables with a Wilcoxon rank-sum test, compared categorical variables using either a chi-square test or Fisher exact test. The significance level was 0.05.

**Results:** Given the racial and ethnic differences between these two offices, an initial control comparison using White and Black chronic ethanol-related deaths indicated no significant difference. The mean age at death from chronic ethanol abuse increased between 2013 and 2018 at both OMI and JCCMO, with a mean age at death of 47.4 years in 2013 and 56.5 years in 2018. Age at death among autopsied cases was lower in New Mexico (47.2 years  $p < 0.007$ ) compared to Jefferson county (51.3 years), possibly due to the younger age at which American Indians died from chronic ethanol abuse (45.3 years,  $p < 0.0001$ ). Regarding sex, women died significantly younger than men ( $p < 0.0001$ ), and this can be explained by the disproportionate number of American Indian females dying young from chronic ethanol abuse (39.4 years).

Autopsy findings demonstrated American Indians had a younger mean age at diagnosis of cirrhosis (42.5 years,  $p < 0.0026$ ), steatosis (42.1 years,  $p < 0.0026$ ), cardiomyopathy (38.7 years,  $p < 0.0016$ ), and jaundice (37.3-year,  $p < 0.01$ ) compared to the rest of the population. Additionally, American Indians comprised a high proportion of cases with varices and cardiomyopathy (35%, and 31%, respectively).

Despite comprising only 8.6% of New Mexico's population, American Indians made up 21.5% of chronic ethanol abuse cases analyzed. Furthermore, American Indian females represented 29% of the female deaths. These dramatic results suggest that factors leading to ethanol abuse and early death within the American Indian population need further investigation.

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### Chronic Ethanol, Alcoholism, Alcohol Abuse

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### H42 A Review of Manner of Death in Denver's Homeless Population (2016–2018)

Shauna Kumar\*, Denver Office of the Medical Examiner, Denver, CO 80204; Meredith A. Frank, MD, Denver Office of the Medical Examiner, Denver, CO 80204; James Louis Caruso, MD, Denver Office of the Medical Examiner, Denver, CO 80204

**Learning Overview:** After attending this presentation, attendees will understand the trends of manner and manner subtypes of death in the homeless population of Denver, CO.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing emerging trends of various causes involving specific drug types, natural disease processes, and multifactorial related deaths in Denver's homeless.

In 2016, 10,550 individuals experienced homelessness in Colorado.<sup>1</sup> The state experienced the third-largest percent increase in homelessness nationally between 2015 and 2016. Homeless individuals are at a greater risk for illness and death than the general population. The experience of being homeless has been found to be an independent risk factor for mortality.<sup>2</sup> Studies show that homeless persons report difficulty accessing health care and experience low rates of outpatient care.<sup>3</sup> Harsh living conditions, such as sleeping outdoors in extreme weather and crowded shelters, increase the risks of exposure to disease, violence, and poor access to proper nutrition. These factors further limit an individual's ability to obtain health care and medications, preventing them from managing health issues. Furthermore, a state of homelessness can result in the exacerbation or development of behavioral health problems.

The Denver Office of the Medical Examiner (DOME) investigates deaths in the City and County of Denver and is responsible for the certification of death. It is involved with the investigation of more than 2,000 deaths and performs approximately 800 autopsies annually. Board-certified forensic pathologists are charged with the examination of a body to determine the cause, manner, and mechanism of death as well as documentation of any injuries or diseases. In this jurisdiction, deaths are categorized by manner, which consist of natural, accident, suicide, homicide, undetermined, and are further subcategorized by cause.

The DOME maintains a searchable database of all deaths occurring in Denver County that are reported to the office. The database was queried for deaths from 2016 to July 2018 in known cases of homeless persons. Specific details of each case were examined, including age, race, and gender of the decedent, as well as place of injury, place of death, any contributing factors, manner, and final cause of death. In cases in which toxicology studies are performed, the specific drug or drugs detected are identified. Toxicological testing is performed primarily on samples of postmortem peripheral blood with occasional substitution of heart or cavity blood. Additional toxicologic studies are performed on urine or antemortem specimens in some cases. Toxicological testing was performed according to internal laboratory protocols with appropriate controls at National Medical Services, Inc, Willow Grove, PA, by means of High Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS).

The total number of homeless deaths in Denver within the specified time was 230. Of those, 114 were accidental (10 traffic related), 80 were natural, 15 were suicide, 11 were homicide, and 10 were undetermined. The total number of homeless deaths in 2016 was 79. Thirty-seven were accidental (4 traffic related), 26 were natural, 5 were suicide, 4 were homicide, and 7 were undetermined. The total in 2017 was 103. Fifty-three were accidental (5 traffic related), 36 were natural, 7 were suicide, 6 were homicide, and 1 was undetermined. The total from January 2018 to July 2018 was 48. Twenty-four were accidental (1 traffic related), 18 were natural, 3 were suicide, 1 was a homicide, and 2 were undetermined. Emerging trends in all three years included deaths ruled as accidental ( $n=104$ , 45%) or natural ( $n=80$ , 34%) as the most common manners. Approximately 50% of the accidental deaths were due to intoxication, showing detection of heroin, cocaine, and methamphetamine or a combination of illicit drugs in combination with prescription drugs or ethanol. Among natural deaths, heart disease with various contributing factors was the most common cause of death at approximately 40%. The majority of cases examined were complete autopsies; however, ( $n=20$ , 8.7%) of these were external examinations only.

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#### Denver, Homeless, Deaths



### H43 Mississippi Organ and Tissue Recovery: A Collaborative Success Story

*Mark M. LeVaughn, MD\**, Office of the Chief Medical Examiner, Pearl, MS 39208; *Anastasia Holobinko, PhD*, Mississippi State Medical Examiner's Office, Pearl, MS 39208; *Taylor Phillips*, Mississippi Organ Recovery Agency, Flowood, MS 39232; *Carrie Deese, BS*, Mississippi Organ Recovery Agency, Flowood, MS 39232; *Kevin Stump, BS*, Mississippi Organ Recovery Agency, Flowood, MS 39232

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**Learning Overview:** After attending this presentation, attendees will better understand organ and tissue recovery rates and trends in a state with a mixed death investigation system (i.e., coroner and medical examiner).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the history of organ and tissue recovery in Mississippi and providing the results of collaborative efforts on recovery rates.

Prior to 2011, the recovery rate of organs and tissues from coroner death investigations throughout the state of Mississippi was extremely low. The abysmal recovery rate was due to a statewide lack of knowledge and education among the county coroners, combined with a lack of cooperation from the non-board-certified pathologists who performed the autopsy examinations. Denials were frequently attributed to interference from the pathologists, who informed the county coroners that organ and tissue recovery would hinder or preclude their ability to determine the cause and manner of death of the potential donor patient. This flawed practice continued throughout the state for nearly 30 years.

In 2011, the Mississippi Department of Public Safety implemented a State Medical Examiner's System and began hiring only board-certified forensic pathologists. Soon thereafter, the State Medical Examiner's Office established communications with the Mississippi Organ Recovery Agency (MORA). The two agencies developed a relationship patterned after the guidelines set forth in the corresponding position paper (National Association of Medical Examiners 2014). The collaboration led to the establishment of a Medical Examiner Referral Process and an on-scene referral process to assist the coroner with potential referrals of patients who would not likely be transported to the hospital or State Medical Examiner's Office.

MORA is one of 58 federally designated organ procurement organizations. The agency utilizes iTransplant<sup>SM</sup>, a web-based clinical management and communications software system that is widely available on hand-held and other electronic devices. The donation and transplantation platform provides rapid information gathering and exchange between multiple agencies including the medical examiner's office.

Over the past several years, the State Medical Examiner's Office and MORA have combined their efforts to educate and train the county coroners regarding the importance of organ and tissue recovery. These efforts have demonstrated to our local coroners and district attorneys that organ and tissue recovery essentially has no negative effect on the determination of cause or manner of death of a potential donor patient. As a result of collaborative efforts and coroner education, MORA has observed nearly a 10% increase in patient referrals in addition to a significant decrease in cases declined by the coroner. It is expected that the recovery rate will continue to increase.

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#### Organ Recovery, Tissue Recovery, Collaborative Effort



### H44 A Death Investigation of Mining Fatalities: The Utah Experience

*Kacy Krehbiel, MD\**, Utah Office of the Medical Examiner, Taylorsville, UT 84129; *Joseph Pestaner, MD*, Utah Office of the Medical Examiner, Taylorsville, UT 84129; *Erik D. Christensen, MD*, Utah Office of the Medical Examiner, Taylorsville, UT 84129

**Learning Overview:** After attending this presentation, attendees will: (1) understand the importance of a thorough death investigation in mining fatalities, (2) be able to describe the impact that autopsy findings have in the subsequent enforcement of safety rules and regulations, and (3) be familiar with the role of outside regulatory agencies in the investigation of these deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by detailing the complexities of the investigation of mining fatalities and highlighting the importance of investigative and autopsy findings in the subsequent regulatory consequences concerning these deaths, as illustrated by a recent case study at the Utah Office of the Medical Examiner (OME).

Mining deaths in the United States, which has active mines in all 50 states, have decreased since the passage of the Federal Mine Safety & Health Act of 1977. This strict liability statute establishes that mine operators may be assessed fines, civil penalties, or even imprisonment for violations of health or safety standards. The number of mining fatalities in the state of Utah has likewise decreased over time, with only one mining disaster (defined as greater than five deaths in an incident) in the new millennium. Death investigation plays a vital role in the investigation and subsequent enforcement of safety rules and regulations. All mining fatalities since 2001 in Utah were reviewed to better understand the investigative challenges in such cases.

Since 2001, there have been 24 mining deaths in Utah that were investigated by the Mine Safety and Health Administration (MSHA), 18 of which were investigated by the Utah OME. The six cases not investigated by the Utah OME were individuals whose bodies were not recovered following the Crandall Canyon Mining disaster of August 2007, in which nine individuals were killed in two separate collapses at this underground coal mine. Investigative issues, such as uncertainty regarding jurisdictional authority, can occur, with overlap between cases falling under the jurisdiction of the MSHA and the Occupational Safety and Health Administration (OSHA). The Utah OME has jurisdiction for all such deaths and works with whichever government agency leads the investigation.

Correlation of autopsy findings with the scene investigation is critical, as exemplified in a recent case at the Utah OME. A 56-year-old male sustained a fatal head injury while installing discharge chutes on the screen deck of a gravel sifter. Witnesses saw the decedent fall backward, but nobody could state what exactly had happened. MSHA's preliminary conclusion, as published in the initial fatality alert report on its website, was that the suspended chute shifted, striking the decedent. This preliminary conclusion was made prior to their review of the autopsy findings. The autopsy revealed significant head injury with two separate significant impact sites that supported an alternative hypothesis, namely that the head was pinched or compressed between two objects. Through subsequent collaboration with MSHA, it was determined that the decedent had most likely been working underneath the suspended chute, in violation of applicable regulations, when his head became pinched between the chute assembly and the surrounding steel structures.

In the investigation of mining fatalities, a thorough understanding of the circumstances surrounding death and correlation with the findings at autopsy is critical. Forensic pathologists should be aware of the role their findings play in the investigation and subsequent regulatory enforcement concerning these deaths.

#### **Mining, Fatal, Investigation**



## H45 Trends in the Certification of Choking-Related Deaths

Joshua Vandeburgh\*, Kalamazoo, MI 49007; Abigail Jacqueline Grande, MPH, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49008; Amanda O. Fisher-Hubbard, MD, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49007

**Learning Overview:** After attending this presentation, attendees will be more familiar with trends in the certification of choking-related deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying risk factors and/or other significant conditions that may contribute to choking-related deaths for complete, accurate death certification.

According to the National Center for Injury Prevention and Control, suffocation, including choking, was the fourth-leading cause of unintentional injury death in 2016.<sup>1</sup> A review of the literature reveals varied etiologies for unintentional death by choking/asphyxiation. Among those greater than 65 years of age, dementia, Parkinson's disease, and pneumonitis were often associated with death by choking on food.<sup>2</sup> In adults, choking is also associated with a variety of conditions, including alcohol intoxication, poor dentition, and dysphagia due to mental illness.<sup>3-5</sup> Although it may seem obvious that individuals with certain conditions, temporary or otherwise, would be more susceptible to choking, it is unclear how these are captured on the death certificate.

The goal of this study is to discern trends in the certification of choking-related deaths, particularly related to risk factors or other conditions that may have made individuals more susceptible to choking. The hypothesis is two-fold: the majority of decedents that die of choking will have a risk factor or contributing cause listed on the death certificate, and/or the decedents will be at the extremes of age.

This study used an electronic database of cases investigated by a medical examiner's office serving multiple counties in Michigan. A search was conducted for "chok\*" in *Part I* and in the *Injury Description* section of the death certificate. The search also included deaths that were categorized as "Asphyxia" with the cause of asphyxia listed as "food/drink." Cases that listed a *Part II* on the death certificate were noted. In addition, *Part I* of the death certificate was surveyed for choking risk factors, including toxicities and neurological disorders.

A total of 71 cases were identified, five of which were incorrectly categorized and one of which had an incomplete death certificate. The remaining 65 decedents ranged in age from 3 to 95 years, with mean and median ages of 62 and 64 years, respectively. Of these cases, the manner of death was certified as accidental in 62 cases; one death was certified as indeterminate due to a family's objection to an autopsy and two deaths were certified as natural. Food or drink was implicated in 57 cases (88%), while an object/other material was implicated in four cases (6%); the causative agent was not specified in four cases (6%). Of the 65 cases, 38 death certificates (58%) documented information in *Part II* and nine (14%) had possible risk factors for choking listed in *Part I b, c, or d*. The majority of *Part II* conditions included neurological/neurodegenerative diseases (17 cases, 45%), while a minority included toxicities (five cases, 13%); in 16 cases (42%), the conditions listed in *Part II* were unrelated to neurological disorders or toxicities. In 8 out of the 65 cases, a neurologic or toxicologic risk factor was listed in *Part I (a, b, c, or d)*. In one case, the medical examiner listed the same risk factor in *Part Ib* and *Part II*.

To summarize, only 30 decedents (46%) had a choking risk factor listed in the cause of death section of the death certificate (*Part I* and/or *II*). These decedents ranged widely in age, although most were 60 years of age or older. To conclude, the way in which choking-related deaths are certified lacks standardization.

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### Choking, Asphyxia, Death Certification



### H46 Cremation Clearance by the Medical Examiner: What Is the Best Method?

*James R. Gill, MD\**, Office of the Chief Medical Examiner, State of Connecticut, Farmington, CT 06032; *Maura DeJoseph, DO*, Office of the Chief Medical Examiner, State of Connecticut, Farmington, CT 06032; *Holly Olko, MA*, Office of the Chief Medical Examiner, State of Connecticut, Farmington, CT 06032

**Learning Overview:** The goal of this presentation is to provide objective data from a single statewide medical examiner system to aid in the determination of whether a physical inspection of a decedent helps detect more unnatural, unreported deaths than just a review of the death certificate/medical records during the process of cremation clearance by the Medical Examiner/Coroner (ME/C).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an evidence-based approach to assessing remains for cremation approval by ME/C jurisdictions.

Numerous jurisdictions require the ME/C to investigate decedents that are to be cremated. Since cremation involves the irreversible destruction of the body, a forensic investigation helps ensure that a previously unreported or undetected unnatural death (homicide, accident, or suicide) is not missed. The process of “clearing” these deaths varies by jurisdiction and ranges from reviewing the death certificate to a physical inspection of the body. With the increasing numbers of cremations and staffing challenges to meet this increased workload, it would be useful to know if one method of cremation investigation is superior to another. The goal of this study is to assess if physical inspection detects more unnatural, unreported deaths than a medicolegal investigation without inspection.

In Connecticut, only an ME can certify homicides, suicides, and accidents. All these known or suspected deaths, as well as all cremation dispositions, must be reported to the Office of the Chief Medical Examiner (OCME). All deaths reported by a funeral home to the OCME for cremation clearance in 2012 and 2016 were reviewed. This review compared the rates of subsequent amendments of the death certificates following two different investigative methodologies performed in each year.

In 2012, physical inspection was the investigative protocol for cremation review. There were 10,367 requests for cremation on non-ME deaths. All but one involved the standard protocol of an OCME-trained medicolegal death investigator performing physical inspections of the remains at funeral homes around the state and reviewing the corresponding death certificate.

In 2016, the cremation clearance protocol was changed to no longer require an inspection of the body but rather a review of the death certificate and other pertinent investigative information as needed. This additional information included a review of the medical records and/or discussions with the family, treating physicians, and or police. In 2016, there were 11,906 of these investigations.

Of the 10,367 reviewed deaths in 2012, there were 86 deaths (0.83%) in which the investigation with physical inspection resulted in an amendment to the cause and/or manner of death (82 accidents, 3 natural deaths, and 1 undetermined). Of the 11,906 reviewed deaths in 2016, there were 153 (1.28%) that required amendment to the cause and/or manner of death (107 accidents, 2 delayed homicides, 2 suicides, 8 therapeutic complications, and 34 natural). The accidents included hip fractures, subdural hematomas, and choking deaths. For the detection of accidents, there was no statistically significant difference between the two groups (chi-square  $\chi^2=0.8119$ ,  $p=.367552$ ). In 2012, four of these 86 decedents had subsequent autopsies at the OCME (3 accidents, 1 natural). In 2016, there were 12 that subsequently underwent autopsy (5 naturals, 5 accidents, and 2 homicides).

For cremation investigations, the manpower and costs of performing physical inspections do not appear justified given the similar detection rates for unnatural deaths among the two groups. Review of the death certificate and associated records without physical inspection detects a comparable number of unnatural deaths as does an investigation with inspection. Without any forensic review of cremations, some ME/C deaths are missed and therefore not properly investigated and certified. Since approximately 30% of deaths in Connecticut do not undergo cremation and do not receive any forensic scrutiny, it is likely that there are unreported ME/C deaths in this non-cremation group.

#### Cremation, Medical Examiner, Investigation



### H47 The Native American Graves Protection and Repatriation Act's (NAGPRA's) Broader Impact: Assessing NAGPRA Implementation Within the Medicolegal System

Megan K. Kleeschulte, MA\*, University of Tennessee, Knoxville, TN 37909

**Learning Overview:** The goal of this presentation is to explore the state of NAGPRA implementation within Medical Examiner/Coroner (ME/C) offices. In doing so, the objective of this research is to aid the medicolegal community in understanding how the law applies to a medicolegal context as well what protocols need to be implemented to be compliant with the law.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by informing attendees of their responsibility to acknowledge that NAGPRA is a federal law and therefore must be followed, as well as that compliance with NAGPRA indicates that ME/C offices, and those working within them, recognize the rights of Native Americans as well as their own role in ensuring those rights are not ignored or violated.

Passed in 1990, NAGPRA was the culmination of efforts by Native Americans, anthropologists, scientists, museum workers, and politicians to provide a legal process for tribes to claim the remains of their ancestors. Section 43 CFR § 10.9 of the law states that universities and museums must prepare an inventory of the items within their possession and return human remains at the request of a lineal descendent, Indian tribe, or Native Hawaiian organization by 1995. An examination of the qualifications that designate a museum for NAGPRA purposes reveals that ME/C offices fall under this definition and are therefore subject to NAGPRA's stipulations and consequences for noncompliance.

As NAGPRA implementation has not been explored or discussed in a medicolegal context to date, a national survey of medicolegal practitioners was distributed to collect data on practitioners' familiarity with the law as well as the presence of disposition protocol for non-forensically significant Native American remains. The survey responses totaled a range of 55–915 cases that are determined to consist of non-forensic, Native American remains each year (this accounts for only a percentage of the total ME/C system). However, the results also indicated that only 44.3% of the survey respondents are familiar with NAGPRA. Additionally, 34.1% of the survey respondents reported that they have an official protocol for the disposition of non-forensic, Native American remains. However, when asked to provide a description of that protocol, not a single response included NAGPRA-compliant disposition procedures. These results indicate that despite the national attention that NAGPRA has received, that law has not succeeded in widespread knowledge and acceptance among the medicolegal practitioners to whom it directly applies.

In order to provide medicolegal practitioners with the knowledge necessary to operate in a compliant manner, and to avoid the fines associated with noncompliance, this research discusses the sections and stipulations of the law that apply to the medicolegal community as well as what NAGPRA-compliant protocol for the disposition of non-forensic Native American remains should consist of. The benefit of the implementation of compliant protocol is two-fold, as it provides a legal mechanism for ME/C offices to transfer these remains out of their control or jurisdiction, avoiding becoming a permanent repository; and acknowledges both the human and civil rights of Native Americans to claim the remains of their ancestors as well as practitioners' roles in ensuring these rights are not violated or ignored.

#### NAGPRA, Implementation, ME/C Office





### H48 Next-of-Kin—May I? Providing Information to an Organ/Tissue Procurement Organization (O/TPO) on Out-of-Hospital Deaths

*Jan M. Gorniak, DO\*, Fulton County Medical Examiner, Atlanta, GA 30312; Charles M. Gammill, BA\*, Lifelink of Georgia, Norcross, GA 30071*

**Learning Overview:** The goal of this presentation is to discuss referring out-of-hospital deaths to an O/TPO. After attending this presentation, attendees will be able to gain consent, if needed, from Next-Of-Kin (NOK) to provide information to an O/TPO.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by possibly increasing the number of life-saving tissues in the community.

Medical Examiners/Coroners are crucial in the donation process. By referring all out-of-hospital deaths to an O/TPO, they can ensure that many citizens in their community are given the opportunity to leave a legacy through the gift of donation. Because the majority of suitable donors fall under medical examiner/coroner jurisdiction, their role in referring out-of-hospital deaths and releasing tissue for donation is one of the most important components in the transplantation process.

Per the Official Code of Georgia Annotated (O.C.G.A) § 44-5-159.2, "... a medical examiner shall release to the procurement organization the name, contact information, and available medical and social history of a decedent whose body is under the jurisdiction of the medical examiner." In 2009, the Fulton County Medical Examiner's Office requested an official opinion from the Fulton County Attorney, specifically, "Is there anything in the law that prohibits us from providing the next-of-kin information to the tissue recovery agency?" The opinion received stated, "Fulton County Medical Examiner **cannot** provide next-of-kin information to the procurement organization that **has not** requested the information." This means that on out-of-hospital deaths, the Fulton County Medical Examiner's Office could not refer cases to the O/TPO.

According to the National Association of Medical Examiners Position Paper, Medical Examiner Release of Organs and Tissues for Transplantation, medical examiners/coroners should have "a goal of 100% referral of out of hospital deaths investigated by medical examiner/coroner offices where last known alive time is within 24 hours." With the county attorney opinion, the Fulton County Medical Examiner's Office was not able to obtain that goal. In November 2016, recognizing the loss of potential donors, the Fulton County Medical Examiner's Office requested a review of the original opinion, specifically asking if the "law prohibits Fulton County Medical Examiner's Office from providing the next-of-kin information to an organ procurement agency such as LifeLink of Georgia®." The current opinion is that "Fulton County Medical Examiner's Office is not prohibited from providing next-of-kin information to LifeLink of Georgia® so long as the next of kin has given their consent prior to releasing this information."

On November 15, 2017, after revising the Organ and Tissue Procurement Standard of Operating Procedure and training medicolegal death investigators, Fulton County Medical Examiner's Office began referring out-of-hospital deaths to LifeLink of Georgia®. Between November 15, 2017, and April 30, 2018, Fulton County Medical Examiner's Office medicolegal death investigators responded to 561 scenes and received next-of-kin consent to share their information in 135 (24%) cases.

#### Donation, Referral, Deaths



### H49 A Novel Adaptation of Electronic Health Records (EHR) for Medical Examiner Use

*Carley J. Holmes\**, Flint, MI 48532; *Kaila Jakubczak, BS*, Hurley Medical Center, Flint, MI 48503; *Brian C. Hunter, MD*, Flint, MI 48502

**Learning Overview:** After attending this presentation, attendees will understand a new way of integrating public health information with clinical information while improving the efficiency of a Medical Examiner's Office (MEO). Epic is an EHR system capable of combining two different health platforms (patient care and postmortem care), providing information sharing capabilities, computer-generated reports, and improved ease of documentation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new information management source for medical examiners that improves access to clinical health information and by exploring the capabilities Epic has to offer the medical examiner community.

Epic is an integrated information management system that can be manipulated to accommodate user's workflow, thereby allowing for improved efficiency in all areas of an MEO: report generation times, processing efficiency, and distribution tracking. The development of a medical examiner module within the Epic system allows for secure sharing of data between clinical care teams and the MEO, both locally and nationally (Epic is widely used throughout the country). Epic's security features limit information sharing to only that which is deemed permissible and appropriate by the involved clinical care system and the involved MEO.

The Genesee County Medical Examiner's Office (GCMEO) in Flint, MI, partnered with Hurley Medical Center, Flint's Level One Trauma Center, to create the first medical examiner/morgue module within Epic, the electronic health record system used by Hurley. The GCMEO had two main objectives when choosing to use Epic: (1) improve the efficiency of the office as a whole, and (2) more seamlessly integrate autopsy data and clinical care information.

There are three main ways Epic can improve efficiency: (1) automatic report generation, (2) specialized notes, and (3) all-in-one storage. The automatic report generation feature can be used as a tool for the National Association of Medical Examiners (NAME) accreditation by pulling together specific information, such as cause of death or manner of death, within a user-specified time range. Specialized notes can be created based on job type (e.g., medical examiner investigators, pathology assistants, medical examiners, etc.); additionally, case reports/notes can be automatically generated based on entered information. Epic provides an all-in-one storage for cases including, but not limited to, images, notes/reports, and past medical history/previous encounters with physicians.

There are three main ways Epic is able to integrate public health information with clinical information: (1) MEO access to clinical health information, (2) clinical physician access to certain MEO information (if permitted), and (3) automatic report generation based on information provided from the MEO. Clinical health information already in Epic for a patient is readily available. Clinical health information may include demographics, diagnoses, surgical history, current medications, lab results, imaging, and encounter notes. Clinical physicians, such as emergency department providers, may be given clearance to view information obtained from performed autopsies on certain patients; case-specific information, such as a trauma report, can be provided in a quick manner through report generation and Epic access. Automatic reports can be generated from the clinical information and the MEO information entered into the system. General information, such as age, sex, and the amount/type of causes of death and manners of death, can be generated for a user-specified time span. Generated reports can be produced from a combination of different information.

In order to quantify efficiency, medical examiner report turnaround time was analyzed from two different time periods: before Epic and with Epic. Additionally, Epic allows for an additional service, a scribe, to aid in report generation at the time of autopsy; therefore, report turnaround time was also analyzed from a period without Epic and a scribe and with Epic and a scribe. It was concluded that report turnaround time was improved (decreased case completion time) with the use of Epic and showed further improvement with the use of Epic and a scribe. Epic provides both enhanced functionality/efficiency and information integration.

#### Electronic Health Records, Medical Examiner, Integration



### H50 Mass Disaster Management and Disaster Victim Identification (DVI) Activities in the 2016 Central Italy Earthquake

Luigi Cipolloni, MD, PhD, Rome 00161, ITALY; Benedetta Baldari\*, Rome, ITALY; Mariantonia Di Sanzo, MD, Rome, ITALY; Livia Besi, Rome, ITALY; Matteo Scopetti, MD, Sapienza University of Rome, Rome, ITALY; Alessia Quattrocchi, Rome, ITALY

**Learning Overview:** After attending this presentation, attendees will better understand the management and identification process of victims after the 6.0 earthquake that struck Amatrice, Italy, on August 24, 2016.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an effective protocol based on the elaboration of an algorithm for the management of dead bodies and on the systematic acquisition of data during corpse processing.

The management of a mass disaster requires a series of fundamental steps concerning the designation of a collection area, transfer, examination, and storage of dead bodies, *in situ* identification, and the return of bodies to relatives for burial. On August 24, 2016, following the earthquake that struck central Italy, a team of forensic pathologists from Sapienza University of Rome were entrusted with managing the disaster.

Upon arrival in Amatrice, a suitable site for the establishment of a collection center was identified. The collection center was divided into an acceptance area, an examination area, and an information center comprising the antemortem section, postmortem data entry, and administrative support. Subsequently, the forensic team developed a cadaver management algorithm based on the World Health Organization guidelines and coordinated, with the local authorities, the transfer of the bodies to the designated center. Upon acceptance, each corpse was attributed an identification number using pre-numbered tags attached to the body and body bag. Each body was then assigned to examiners for data collection. The examiner's team was composed of forensic pathologists and police officers from the Carabinieri scientific investigation division and the scientific police. At least four examiners were assigned to each body to conduct a systematic examination. Findings were documented through compiling data collection forms and taking photos of the body, clothing, accessories, and other personal items. Only identified bodies were sent to be buried in Amatrice cemetery or in other places for non-residents. The bodies without identification were momentarily placed in refrigerated tents awaiting identification. In cases in which identification did not occur within a reasonable time, burial was ordered for hygienic and health reasons after fingerprints collection and DNA sampling.

From August 24–28, 2016, 244 bodies were received at the collection center. Most of the 244 bodies examined were complete bodies (235, 96.3%), while in a few cases, the bodies were dismembered (9, 3, 7%), with the loss of anatomic parts such as the head, ears, limbs, feet, and fingers. Sex was recognizable in all 244 bodies with a male- (98, 40%) to-female (146, 60%) ratio of 1.48.

The causes of death of the 244 subjects examined were classified, according to the *International Classification of Disease, 10<sup>th</sup> Edition (ICD-10)* Lists for Tabulating Mortality Statistics, into crushed chest (124, 51%), confinement or entrapment in a low-oxygen environment (66, 27%), fractures involving multiple body regions (27, 11%), fractures of the skull and facial bones (20, 8%), and fractures of the cervical spine (7, 3%).

Most of the bodies (227, 93%) were directly identified through clothes, personal effects, marks, photographs, and comparison between antemortem and postmortem data. In a minority of cases (17, 7%) it was necessary to take samples for DNA analysis. At the end of the operations, all the deceased had been identified and returned to relatives.

Identification of mass disasters victims is a complex activity because of the emergency conditions in which the operations take place and the conditions of conservation in which the bodies are found. In this context, the role of forensic pathologist assumes extreme importance in the coordination of the multidisciplinary team involved in the post-disaster management of victims. The experience proposed demonstrates, albeit in a small-scale context, that a standardized protocol focused on identification is highly effective, reducing the use of more advanced methods.

#### Mass Disaster, Earthquake, Identification



## H51 Acute Myocarditis Following PD-1 Inhibitor (Nivolumab) Treatment for Lung Carcinoma

Peter Houston, MD, Charleston, SC; Angelina I. Phillips, MD\*, Medical University of South Carolina, Charleston, SC 29425

**Learning Overview:** After attending this presentation, attendees will: (1) understand the pathophysiology of Immune Checkpoint Inhibitors (ICIs), (2) become aware of myocarditis as a severe immune-related adverse event, and (3) include immunomodulatory treatments in the differential diagnosis of acute myocarditis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the increasing need to include ICIs in the differential diagnosis of acute myocarditis when evaluating a patient's anticancer regimens.

Nivolumab is a monoclonal PDL-1 inhibitor in the class of ICIs used in the treatment of several malignancies, including non-small cell lung cancer, renal cancer, and melanoma.<sup>1,3</sup> Common reported treatment-related adverse events of immunomodulators include fatigue, decreased appetite, rash, diarrhea, and arthralgia, including treatment-related autoimmune effects in sites such as skin, kidney, lungs, brain, endocrine organs, gastrointestinal tract, and, more recently described, cardiovascular system.<sup>1,2,4</sup> Over-expression of inhibitory checkpoint molecules, such as Programmed cell Death Ligand 1 (PDL-1) allows cancer cells to prevent the body's natural immune response from activating CD8 T-cells, allowing the cancer cells to proliferate, escaping cytotoxic cell mediated death.<sup>2</sup> ICIs work by blocking PDL-1, which allows the CD8 T-cells to become active and kill cancer cells, although not without some notable side effects. Severe cases of immune-related adverse events have been well documented. Of these, approximately 0.06% to 0.27% of patients can experience immune-related myocarditis that can lead to arrhythmias, heart failure, and death due to cardiogenic shock.<sup>2</sup>

This presentation describes a fatal case of fulminant myocarditis after treatment with nivolumab in a 76-year-old man with metastatic lung adenocarcinoma. The patient presented with chest discomfort while at the infusion clinic; he reported experiencing some nausea and vomiting, shortness of breath, and dizziness approximately five days prior. Examination revealed atypical Electrocardiogram (EKG) findings, including ventricular tachycardia with apparent Atrioventricular (AV) dissociation and S-T segment elevations concerning for S-T-Elevation Myocardial Infarction (STEMI) with serum troponin elevations suggestive of a myocardial infarction; the patient died within one day of admission. Postmortem examination was requested by the clinical team with suspicion of metastasis to the AV node due to EKG findings.

At autopsy, the gross examination of the heart was unremarkable except for evidence of coronary atherosclerosis and focal pallor in the interventricular septum. Histologic examination revealed a fulminant myocarditis with infiltration of the myocardium by lymphocytes, numerous neutrophils and eosinophils associated with marked myocyte necrosis, and interspersed large atypical appearing cells. The large atypical cells were concerning for metastatic tumor; however, immunohistochemical workup ruled out carcinoma. Infective myocarditis workup performed at the Center for Disease Control could not identify a specific infectious etiology. Ruling out the presence of metastatic tumor and common infectious causes, the final diagnosis favored the immune check point inhibitor treatment as the etiology of the fulminant myocarditis.

Multiple immune checkpoint inhibitors are on the market for use in the anticancer regimens of a variety of cancer types, which has markedly increased the number of patients receiving these therapies. This report adds to the cases of acute myocarditis presenting after PD-1 inhibitor treatment and highlights the need to consider a broader differential in myocarditis presenting in patients with cancers treated by immunomodulators.

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### Cardiac Pathology, Myocarditis, PD-1 Inhibitors



### **H52 Arrhythmogenic Atrioventricular Node Fibrosis—An Unexpected Cause of Death in the Young: Regarding Two Autopsy Cases**

*Paulina Henry, MD, Cliniques Universitaires saint-Luc, Brussels 1200, BELGIUM; Gregory Schmit, MD\*, Service de Médecine Légale, Brussels 1200, BELGIUM; Julie Lelotte, MD, Cliniques Universitaires saint-Luc, Brussels 1200, BELGIUM; Jessica Vanhaebost\*, Brussels, BELGIUM*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### H53 Madelung's Disease: A Rare and Benign Life-Threatening Condition

*Helena D. Corro, MD\**, Portuguese National Institute of Legal Medicine, Coimbra, Beira Litoral 3000, PORTUGAL; *Carla Carreira, Largo da Sé Nova, Coimbra, PORTUGAL*; *Rosa H. Gouveia, PhD, Portuguese National Institute of Legal Medicine, Coimbra 3000-213, PORTUGAL*; *Ana S. Coelho, MSc, National Institute of Legal Medicine, Coimbra, PORTUGAL*; *Joao E.S. Pinheiro, PhD, MD, Instituto Nac Medicina Legal e Ciências Forenses, Coimbra 3000-213, PORTUGAL*; *Francisco Corte-Real, PhD, Center Branch, Coimbra, PORTUGAL*

**Learning Overview:** After attending this presentation, attendees will understand about a rare and benign condition called Madelung's Disease, which can mimic head and neck malignancy and add an asphyxial component to the cause of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing this rare disorder of unknown etiology, also called Launois-Bensaude Syndrome and Multiple Symmetric Lipomatosis, and provide tools for its diagnosis. The forensic implications to the cause of death are also relevant.

Madelung's disease is characterized by a prominent and symmetric deposition of non-encapsulated adipose tissue, preferentially on the upper region of the body. Symptoms depend on the affected body area. With only 200-300 cases described worldwide until 2015 and an incidence of 1/250,000, it is more frequently observed in middle-aged men with Mediterranean ancestry, strongly associated to chronic alcoholism. Forensically, the excess of fat accumulation around the neck is the most striking feature, because it may compress the aero-digestive tract, and/or the lipomatosis aggregates may undergo malignant transformation. In such cases, surgical excision or liposuction with removal of the entire lesion could be attempted.

Reported here is a case of a 48-year-old man with a history of heavy alcoholism, oligophrenia, and Madelung's Disease admitted to a mental health department for alcohol detoxification and posterior surgical treatment, who died suddenly when sleeping. The patient exhibited a good general condition, BMI of 28.7, and large adipose neoformations on the submandibular, cervical, supraclavicular regions, and upper arms. The patient had previously been submitted to surgery for this disorder.

The autopsy showed, apart from the above-mentioned external lesions, an enlarged amount of anomalous deposition of adipose tissue surrounding the aero-digestive tract with compression of laryngeal structures, although still permeable. Anatomico-pathological examination revealed benign subcutaneous and soft tissue lipomatosis that compressed and deformed the local structures, affecting their natural functions (as shown by marked distention of alveolar spaces and pulmonary infection). Toxicology was negative for alcohol, revealed anti-epileptic, neuroleptic, and benzodiazepines in therapeutic concentrations. The cause of death was acute bronchopneumonia with a probable contribution of the cervical lipomatosis to respiratory compromise.

Forensic pathologists should be aware of the characteristic appearance of this unique and rare disorder, scarcely described in forensic literature, as an important clue to recognize associated risk factors, such as alcoholism, hyperglycemia, hyperlipidemia, diabetes, and polyneuropathy. It is also important to evaluate, for each case, its role in an eventual asphyxial death.

#### **Madelung's Disease, Multiple Symmetric Lipomatosis, Asphyxial Deaths**



### H54 Disseminated Miliary Staphylococcal Infection: A Case of Pyomyositis and Bacterial Myocarditis in a Prisoner

Ariana Pape, BA\*, Norfolk, VA 23508; Wendy M. Gunther, MD, Office of the Chief Medical Examiner, Tidewater District, Norfolk, VA 23510-1046

**Learning Overview:** After attending this presentation, attendees will be able to: (1) recognize the signs and symptoms of disseminated staphylococcal infection, (2) differentiate bacterial from fungal meningitis, and (3) familiarize themselves with the tissues most likely to be invaded by disseminated staphylococcus aureus.

**Impact on the Forensic Science Community:** This presentation will impact attendees by: (1) increasing clinical suspicion of disseminated pyomyositis in severe sepsis of unclear source, and (2) suggest alternate testing beyond routine culture and radiology, such as investigation of the heart and skeletal muscle by biopsy, ultrasound, and magnetic resonance imaging.

Fatalities due to disseminated staphylococcal infection are relatively rare. Most staphylococcal infections do not disseminate and are focused in a specific area of the body. Here is reported a case of a 39-year-old male prisoner who died of disseminated miliary staphylococcal infection. He had a history of chronic drug addiction with repeated overdoses and had previously been treated in the hospital for an overdose two months before imprisonment. He became ill in jail after the first ten days of imprisonment, spent three days in the medical isolation ward, and was transferred to the hospital for lethargy, confusion, and tachycardia with decreasing blood pressure. After a negative drug screen, the differential diagnosis on admission was focused on severe sepsis of unclear origin. Meningitis was suspected to be due to cryptococcal infection, due to a positive India ink test, but that was later ruled out due to antigen testing. He was also diagnosed with a urinary tract infection, sepsis, and pneumonia, all due to methicillin-resistant *Staphylococcus aureus*. He died in the hospital before the origin of the meningitis was able to be determined.

On autopsy, external examination showed petechial hemorrhages and severe jaundice. The petechial hemorrhages were in the sclerae, oral mucosa, and skin and corresponded to a markedly reduced platelet count due to diffuse intravascular coagulation. Internal examination identified disseminated pustules in a miliary pattern throughout skeletal muscle and the myocardium, along with bilateral consolidated pneumonia, hepatomegaly, splenomegaly, and splenic infarcts. The disseminated miliary pustules were found in the pectoral, abdominal, and strap muscles, myocardium, lungs, tongue, and jejunal wall. Several of the pustules had abscess cavities, and the lungs were the most severely affected. The Cerebrospinal Fluid (CSF) gram stain demonstrated innumerable gram-positive cocci without fungal organisms; multiple blood and urine cultures grew *Staphylococcus aureus*. The cause of death was determined to be disseminated miliary staphylococcal infection despite appropriate antibiotic therapy.

The literature includes one report of eight autopsy cases of fatal pyomyositis due to *Staphylococcus aureus* infection.<sup>1</sup> Six out of the eight cases involved recent trauma to the area of infection, and the pustules were most frequently found in the trunk, shoulder girdle, and thigh muscles. Besides the pyomyositis, cirrhosis and fatty liver were the most frequent autopsy finding, and death was due to sepsis in all cases. This case confirmed the skeletal muscle pattern of pyomyositis and adds cardiac involvement; there was no recent trauma. A second case report on *Staphylococcus aureus* myocarditis, reporting a 44-year-old male on methotrexate who was diagnosed with *Staphylococcus aureus* myocarditis associated with a left ventricular apical thrombus, noted that it is seen most frequently in immunocompromised persons.<sup>2</sup> That patient was treated with antibiotics and made a complete recovery. The case presented here was reportedly HIV negative and had no known immunosuppression; his disseminated miliary staphylococcal infection with pyomyositis and cardiac involvement was not suspected prior to autopsy.

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#### Pyomyositis, Bacterial Myocarditis, Disseminated Infection



### H55 Deaths Due to Cardiomyopathy of Unknown Etiology in Children and Young Adults

*Serenella Serinelli, MD\**, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210; *Ponni Arunkumar, MD*, Cook County Medical Examiner's Office, Chicago, IL 60612; *Steven M. White, MD, PhD*, Cook County Medical Examiner's Office, Chicago, IL 60612; *Lorenzo Gitto, MD\**, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210

**Learning Overview:** After attending this presentation, attendees will better understand the autopsy findings of deaths due to cardiomyopathy of unknown etiology in children and young adults in the Cook County Medical Examiner's Office in Chicago, IL.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating cases of sudden, unexpected death in which the hearts were abnormal (either grossly or microscopically), but there was no known underlying etiology for the cardiomyopathy.

Cardiomyopathies are a heterogeneous group of diseases of the heart muscle that can lead to heart failure, cardiac arrhythmia, and sudden death. Although there are many etiologies for cardiomyopathies, they typically manifest as an enlarged heart or a heart with normal weight with microscopic pathology (fibrosis, inflammation, etc.). Since cardiomyopathy is not a specific diagnosis, this study has attempted to determine the underlying etiologies in our population. Diseases such as hypertension, obesity, chronic alcoholism, and genetic abnormalities can all cause cardiomyopathies and have similar gross and microscopic findings. This project focused on cases of cardiomyopathies in which no underlying etiology could be found through medical history or autopsy.

The files of the Cook County Medical Examiner's Office in Chicago, IL, were searched for cases of subjects between 1 and 40 years of age with "cardiomyopathy" as a cause of death, from January 2013 to June 2018. Cases were reviewed for age, sex, race, cause and manner of death, medical history, and autopsy findings.

In total, 140 cases were identified: 116 cases (83%) with cardiomyopathy due to hypertensive cardiovascular disease, 1 case with cardiomyopathy due to obesity, 1 case with cardiomyopathy due to chronic alcoholism, 2 cases with cardiomyopathy due to alcoholism and obesity in combination, and 20 cases (14%) with an unknown etiology, which is the group examined for this study.

Regarding the 20 cases with a cardiomyopathy of unknown etiology, 16 were males and 4 females; 15 were African American and 5 Caucasian. Ages ranged between 14 and 40 years old: two decedents were <20 years old, eight ranged between 21-30 years, and ten ranged between 31-40 years. The manner of death was determined to be natural in all cases.

Cardiomegaly was present in 16 out of 20 decedents (80%) with heart weights ranging between 376 grams and 840 grams. Additional gross pathological changes were present in 15 out of 20 cases (75%), consisting mostly of dilated ventricles, hypertrophic ventricles, and myocardial scarring.

All 20 cases showed microscopic pathology consisting of the following changes: fibrosis (17 cases), cardiac myocyte hypertrophy (13 cases), interstitial chronic inflammation (10 cases), and interstitial adipose tissue (5 cases). In 4 out of 20 cases (20%), there were no gross pathological changes, but histology showed one or more of the above-listed abnormalities.

Regarding medical history, 10 out of 20 cases had other associated diseases, with the most common being obesity, asthma, and fatty liver. Obesity was present in 6 out of 20 cases (BMI ranged between 32 kg/m<sup>2</sup> and 35.3kg/m<sup>2</sup>). In cases in which obesity was considered as the etiology of cardiomyopathy, BMI ranged between 40kg/m<sup>2</sup> and 46kg/m<sup>2</sup>.

Genetic testing is being offered to decedents' families to search for an underlying etiology and counsel families. To date, this study has results from 3 out of 20 cases. So far, no pathogenic mutations have been identified. Genetic variants of uncertain significance have been identified, including missense variants of genes PRKAG2, DOLK, DMD, NEBL, PDLIM3, and DSG2. At this point, it is unclear how these variants may contribute to the development of cardiomyopathies. Therefore, in all the autopsy reports, it was recommended that first-degree (immediate) family members undergo cardiovascular screening and possible genetic evaluation by experienced cardiologists and geneticists to diagnose and prevent sudden death in surviving family members.

This study shows that in children and young adults, there are a significant number of decedents with cardiomyopathy with no known etiology after a complete autopsy and review of medical records. In some cases, the heart may appear grossly normal and show only microscopic evidence of pathology. Screening for cardiovascular disease and possible genetic testing should be performed in immediate family members of a child or young adult dying from a cardiomyopathy of unknown etiology.

#### Cardiomyopathy of Unknown Etiology, Sudden Death, Genetic Testing





### H56 Alveolar Capillary Dysplasia With Misalignment of the Pulmonary Veins: Histopathology and Applicability to Autopsy

Rhonda M. Mittenzwei, MD\*, Duke University Health System, Durham, NC 27710; Elizabeth Pavlisko, MD, Duke University Medical Center, Durham, NC 27710; Laura Hale, MD, PhD, Duke University Medical Center, Durham, NC 27710

**Learning Overview:** After attending this presentation, attendees will be able to: (1) identify the pathognomonic histologic features of a rare lung development disorder, Alveolar Capillary Dysplasia with Misalignment of the Pulmonary Veins (ACD-MPV); and (2) recognize its clinical presentation and understand confirmatory testing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by elucidating a rare disease process that may present as unexplained cardiopulmonary arrest in an infant and providing diagnostic criteria that can help make a diagnosis.

Herein, a case of ACD-MPV is presented in which this diagnosis was not initially suspected clinically. The patient presented with respiratory failure within hours of birth, with subsequent development of pulmonary hypertension refractory to treatment. He received near-constant ventilator support and was put on Extracorporeal Membrane Oxygenation (ECMO) twice during his three weeks of life. Several hours after his second ECMO decannulation, he acutely decompensated with systemic hypotension and pulmonary hypertension requiring the use of vasopressors and fluid resuscitation. Infection was suspected clinically due to a rising white blood cell count in the context of hypotension, but all cultures were negative. The patient's ultimate demise was precipitated by an acute hypoxic event; his family opted to have an autopsy performed. Gross autopsy findings included left pulmonary artery stenosis with ostial stenosis, pulmonary trunk dilation, and right ventricular hypertrophy. Light microscopy revealed a decreased number of pulmonary capillaries and increased distance from alveolar epithelium in conjunction with pulmonary veins and pulmonary arteries in the same adventitial sheath. Genetic testing performed on the patient identified a c.691\_698delGCGGCGGC frameshift mutation in the *FOXF1* gene.

ACD-MPV is a rare but generally fatal developmental disorder of pulmonary vasculature. Genetic variations of the *FOXF1* gene, including heterozygous point mutations, deletions, and mutations of an upstream enhancer region have been identified in patients with ACD-MPV, and mutations in *FOXF1* have been shown to cause similar pulmonary vascular abnormalities in mice. However, pedigree analysis suggests that mutations in other still unknown gene(s) may also result in ACD-MPV. The initial presentation of this disorder typically involves neonatal respiratory distress and pulmonary hypertension, both of which are refractory to supportive measures. Respiratory distress typically occurs within 24–48 hours after birth and may be accompanied by central cyanosis and tachypnea. The clinical presentation may be exacerbated by a concomitant hypoplastic left heart. The *FOXF1* gene is also involved in gastrointestinal tract development, and malrotation or intestinal atresia may be identified alongside lung abnormalities. Rarely, genitourinary malformations are identified.

Histopathology is considered the gold standard for diagnosis of ACD-MPV. Tissue may be collected via a lung biopsy, if tolerated, but is often collected at the time of autopsy. Five histopathologic findings are diagnostic of ACD-MPV: immature lobular development; decreased number of pulmonary capillaries located away from alveolar epithelium; thickened alveolar septa; arteriolar and arterial changes, including medial hypertrophy and muscularization; and malposition of pulmonary veins within the same adventitial sheath as pulmonary arteries. In up to 30% of cases, lymphangiectasis may also be identified.

This case report highlights the importance of autopsy in elucidating a disease process that was not initially suspected clinically. It is important for pediatric critical care teams to include ACD-MPV in the differential diagnosis of patients with severe and refractory respiratory failure and pulmonary hypertension. Definitive diagnosis can be made by lung biopsy, if tolerated. Identification of an inactivating *FOXF1* mutation can confirm the diagnosis, but absence of such a mutation cannot definitively rule out ACD-MPV. Prompt antemortem diagnosis may allow the development of treatments that can prolong survival, as no effective treatments for ACD-MPV exist currently, and may assist families in coping with their child's prognosis. In the broader context of forensic autopsies, the diagnosis of ACD-MPV may be identified histopathologically within the context of an otherwise unexplained cardiopulmonary failure in a term neonate.

#### Alveolar Capillary Dysplasia, Misalignment of Pulmonary Veins, Histopathology



### H57 Exacerbation of Traumatic Brain Injury in the Presence of Diabetes Mellitus: An Experimental Study of Mice

*Kazuhiko Kibayashi, MD\*, Tokyo Women's Medical University, Tokyo 162-8666, JAPAN*

**Learning Overview:** After attending this presentation, attendees will understand the relationships between pre-existing natural diseases and traumatic injuries and the possible exacerbation of injuries after experiencing trauma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing evidence that diabetes mellitus is a possible condition that contributes to death due to Traumatic Brain Injury (TBI).

TBI can occur as a result of falls, traffic accidents, and blows. An assessment of severity is required to determine the prognosis and elucidating cause of death. It is known that age is a risk factor for TBI. Elderly people sometimes have diabetes mellitus as a pre-existing co-morbid disease. Diagnosis of a coexisting or pre-existing condition that contributes to underlying cause of death is required in completing the cause-of-death statement. It was supposed that TBI is exacerbated by pre-existing diabetes mellitus due to microcirculation dysfunction and prolonged inflammation. This study investigated the change in brain function and extent of brain contusion after TBI in diabetic and non-diabetic mice.

Ten-week-old male KKAY (type 2 diabetic) and C57BL/6J (non-diabetic) mice were used in this study. A Controlled Cortical Impact (CCI) device was used to induce TBI in each mouse (CCI model). Sham-operated (craniotomy without impact) and naive (did not undergo any operation) animals were used as controls. This study measured the volume of brain contusion using Magnetic Resonance (MR) imaging, which was performed using the Bruker® Icon 1T MR imaging system. This study then assessed changes in nerve function using the Neurological Severity Score (NSS) for motor function and cognitive deficits, learning and memory using the Morris Water Maze (MWM), depression/helplessness using the Forced Swim Test (FST), and motor function using Beam Walking (BW). These experiments were performed from 1 to 112 days after surgery ( $n=4-10$  per timepoint for each group).

Brain contusion was produced in the ipsilateral cerebral cortex of the diabetic and the non-diabetic mice. Compared with those in the non-diabetic mice, the diabetic mice had significantly higher volumes of brain contusion based on the MR imaging and significantly greater deterioration in NSSs after TBI. There were no significant differences in MWM, FST, or BW performances between the diabetic and non-diabetic mice. The results of this present study suggest that pre-existing diabetes is a factor that worsens TBI. Exacerbation of TBI should be monitored in patients with diabetes mellitus. This experimental study also suggests that coexisting diabetes mellitus is a condition that contributes to death due to TBI.

#### **Cause of Death, Diabetes Mellitus, Traumatic Brain Injury**

### H58 Primary Cardiac Tumor as a Cause of Sudden Death: A Report of a Rare Case of Atrial Lipoma

*Stefano D'Errico, MD, PhD, Department of Legal Medicine, Lucca 55100, ITALY; Diana Bonuccelli, MD, Department of Legal Medicine, Lucca, ITALY; Alberto Mandoli, MD, Department of Legal Medicine, Lucca, ITALY; Massimo Martelloni, MD, Department of Legal Medicine, Lucca, ITALY; Francesca Maglietta, MD\*, Department of Forensic Pathology, Foggia 71122, ITALY*

**Learning Overview:** The goal of this presentation is to present the case of a sudden undiagnosed cardiac lipoma of the right atrium.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing fatal cardiac rhythm disturbances caused by an atrial tumor infiltrating the free wall of the right atrium and the role of primary cardiac tumors as a cause of sudden death.

Primary tumors of the heart are rare, and the incidence varies between 0.0017 and 0.19% in unselected autopsy studies. Among these, more than 70% are benign, mainly myxomas. Other benign tumors are lipoma, papillary fibroelastoma, rhabdomyoma, and fibroma. It is extremely rare, with a reported incidence of approximately 10%–19% among primary tumors of the heart and pericardium. Histopathologically, cardiac lipoma can be classified into two types: lipomatous hypertrophy of the interatrial septum and true lipoma. Approximately 50% of cardiac lipomas arise subendocardially, 25% subepicardially, and 25% from the myocardium. The most common chambers involved are left ventricle and right atrium with a prevalence between 40–60 years of age without sex predominance and extremely variable in size. Patients with cardiac lipoma are usually asymptomatic and discovered incidentally; symptoms reported are fatigue, dyspnea, palpitation, syncope, and even chest pain, which are frequently a result of coronary artery or cardiac conductive system involvement. Tumors in the subepicardial region can create a mass effect on nearby structures. They can cause angina if they compress the coronary arteries, or they can reduce systolic function by compressing on the left ventricle. Tumors in the myocardium can infiltrate the electrical circuit and be a nidus for arrhythmogenesis. Depending on the chamber involved and the size of the mass, they can cause obstruction of flow and congestive heart failure.

Cases of sudden unexpected death attributed to myocardial tumors have been poorly described in forensic and clinical literature; in these cases, cardiac neoplasms cause atrioventricular or intraventricular conduction disorders, which are manifested by arrhythmias, interfering in the cardiac dynamic and leading to sudden death. It has been calculated that 0.0025% of all cardiovascular deaths may be sudden death caused by primary cardiac lesions and 0.01%–0.005% of all sudden deaths could be due to primary cardiac tumors and 0.06% of cardiovascular deaths among the 0 to 34-year-old population may be the result of sudden death caused by a primary intracardiac tumor. These data indicate that primary cardiac lesions are uncommon, yet potentially lethal. Owing to the rarity of these lesions, it seems likely that many practicing autopsy pathologists will never encounter a primary cardiac lesion. It is also expected that several primary cardiac tumors causing sudden death will be missed each year because an autopsy is not performed.

**Case Report:** Presented here is the case of a 67-year-old man who was found unconscious at home and immediately taken to the emergency department of the local hospital. He had a history of hyperthyroidism and hyperlipidemia. One hour after recovery, clinical conditions improved, he was without pain and oriented (GCS 15); normal cardiac function was recorded at Electrocardiogram (ECG), and neurological examination was unremarkable. Two hours after recovery, he suddenly and unexpectedly died. A hospital autopsy was performed the day after death. External examination was unremarkable. Gross examination revealed an encapsulated tumor (40mm x 30mm x 30mm) in the external layer of the right atrium free wall. The section showed a yellowish and elastic solid mass. The heart was normal in size and shape and the coronary arteries were unremarkable. A heart examination was performed according to the inflow-outflow method; no further pathological findings were attributable to the heart except for the presence in the subendocardial wall of the right atrium of three yellowish nodular buttons 1cm in diameter. Mild pulmonary edema was also recorded, with white foam on the main bronchi. The histologic study was completed using formalin-fixed paraffin embedded tissue sectioned at 4mm and stained with hematoxylin–eosin. Microscopic findings were suggestive for a right atrial lipoma infiltrating through the atrium wall with mild intracardiac involvement. The diagnosis was confirmed by immunostaining that revealed immunopositivity for S100. Based on morphological and microscopic findings, a sudden arrhythmogenic cardiac death related to an atrial rhabdomyoma was diagnosed.

#### Sudden Death, Cardiac Lipoma, Fatal Arrhythmia



**H59 WITHDRAWN**



### H60 Massive Portal Vein Thrombosis as a Rare Cause of Unexpected Death in a Non-Cirrhotic Patient

*Stefano D'Errico, MD, PhD, Department of Legal Medicine, Lucca 55100, ITALY; Sara Niballi, MD, Department of Legal Medicine, Lucca, ITALY; Giuseppe Davide Albano, MD\*, Foggia 71121, ITALY; Massimiliano Esposito, MD, University of Catania, Catania 95123, ITALY; Marco Conti, MD, Department of Legal Medicine, Lucca, ITALY*

**Learning Overview:** The goal of this presentation is to present the case of an unexpected death in a non-cirrhotic patient with acute abdominal pain of recent onset due to a massive Portal Vein Thrombosis (PVT).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that a common presenting symptom, such as abdominal pain, in a young, healthy adult can be a manifestation of a rare diagnosis such as PVT, supporting the relevance of the hospital autopsy as an instrument of audit in the practice of emergency medicine.

PVT as the etiology of abdominal pain in an otherwise healthy young adult is an uncommon occurrence and is capable of leading to serious adverse short- and long-term events in the affected patients. While the occlusion of the extrahepatic portal and splenic vein can provoke complications mainly related to portal hypertension, mesenteric vein obstruction shows a high rate of complications and mortality due to intestinal infarction. The occurrence of this condition is probably underestimated given the fact that the clinical appearance can vary from asymptomatic patients incidentally diagnosed to patients with severe complications. The underlying cause of PVT includes malignancy, chronic liver diseases, local inflammatory processes, systemic disorders, including myeloproliferative disorders, and thrombophilia.

In a high percentage of patients, two or more risk factors are present. In population studies, the cohort of patients with occlusion in the portal tributary system can be divided into three similarly large subcohorts of major etiologies: malignant thrombosis mainly due to gastrointestinal, hepatobiliary, or pancreatic cancer; chronic liver diseases, especially liver cirrhosis with portal hypertension; and non-malignant, non-cirrhotic PVT. While the underlying disease determines the natural history and outcome of patients in the case of malignant thrombosis, the two other groups of patients are characterized by an outcome influenced by PVT. Furthermore non-malignant, non-cirrhotic PVT has often been described as an entity of its own, leading to non-cirrhotic portal hypertension, which represents more of a consequence than a complication of another disease. It has been estimated that in non-cirrhotic PVT patients, thrombophilic states account for approximately 40% to 60% of PVT cases, and local factors are thought to be the causative factor in 10% to 50%. In about one-third of patients, the etiology is unknown. The site, extent, chronicity, and course of thrombosis determine clinical presentation as well as complications in affected patients. While partial PVT is usually discovered incidentally by routine diagnostics and remains clinically silent, the complete occlusion of the vein (90%–100% of the lumen) is associated with abdominal and/or lumbar pain characterized by sudden onset or progressive development over the course of a few days. Acute and complete thrombosis is usually associated with intestinal congestion and occasionally with non-sanguineous diarrhea. The most feared complication is intestinal infarction with a mortality of 20%–60%, leading to extended resections with a high risk of postoperative complications. In contrast to intestinal congestion, infarction often presents with persistent pain, hemochezia, guarding, contractures, ascites, or multiorgan failure with metabolic acidosis. This complication is usually found when the mesenteric veins are involved.

**Case Report:** A 61-year-old man reached the emergency department of the local hospital complaining of onset of acute abdominal pain for two days. Hypotension (80/60mmHg) and bradycardia (55bpm) were detected when he suddenly collapsed. Resuscitation maneuvers were attempted, unsuccessfully. Hospital autopsy was performed the day after death to investigate cause of death. Diffuse mesenteric ischemia was observed due to massive PVT extended to splenic and mesenteric veins. Liver was increased in volume (32cm x 20cm x 10cm), non-cirrhotic, with mild steatosis. Histological assessment of thrombus samples with immunohistochemistry was also performed to establish chronological stage. Audit and mortality and morbidity review with clinicians was performed after postmortem investigation.

#### Portal Vein Thrombosis, Hospital Autopsy, Mesenteric Ischemia



**H61 WITHDRAWN**



### H62 Asbestos Fiber Burden in the Lungs of the Deceased From Asbestos-Related Diseases (ARD): An Epidemiological Study and Scanning Microscope/Energy Dispersive Spectroscopy (SEM/EDS) Analysis

Silvia D. Visona, MD\*, University of Pavia, Pavia 21100, ITALY; Silvana Capella, PhD, University of Torino, Torino, ITALY; Elena Belluso, University of Torino, Department of Earth Sciences, Torino 10125, ITALY; Simona Villani, PhD, Department of Public Health, Experimental, and Forensic Medicine, Pavia, Lombardia 27100, ITALY; Federica Manzoni, University of Pavia, Pavia 27100, ITALY; Yao Chen, MD, University of Pavia, Pavia 27100, ITALY; Giorgio Ardissino, MD, Department of Forensic Medicine, Pavia, ITALY; Francesca Russo, Pavia, ITALY; Antonio M.M. Osculati, MD, University of Pavia, Pavia 27100, ITALY

**Learning Overview:** The goal of this presentation is to investigate a large series of deaths due to ARD, through an extensive epidemiological study and, in a smaller group of individuals, by assessing the fiber burden in the subjects' lungs using SEM/EDS to clarify the role of asbestos in causing human diseases, primarily mesothelioma.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing novel insights regarding the impact of asbestos on human health using biological samples collected during forensic autopsies.

ARDs are often brought to the forensic pathologist's attention due to the high mortality of mesothelioma and lung cancer and to the complex legal implications, related to the responsibility of the manufacturers. Even though the use of asbestos has been banned in the United States, as well as in most European countries, the widespread production and use of asbestos have caused unprecedented human suffering and still represents a major public health problem all over the world. Note that in many countries, the mining and use of asbestos is still allowed (e.g., Canada, Russia, China). The latent onset of disease from exposure has led to a catastrophic epidemic and a continuing onslaught because of people being exposed decades ago. Despite the relationship between asbestos and mesothelioma, lung cancer, and asbestosis being well documented, many issues concerning the etiopathogenesis of ARD, especially mesothelioma, are still debated. For example, the role of fiber type and dimension (length and thickness), the importance of exposure dose, and the dose-response effect are still questioned.

This retrospective study included 188 subjects who died from ARDs (mesothelioma, lung cancer, and asbestosis) during 2000–2017 in the area around Broni, Italy, where an important asbestos cement factory had been active from 1932 until 1993. A forensic autopsy was performed in each case, during which the entire lungs were collected and formalin-fixed. The main objective consisted in analyzing the records, including the clinical files, the autopsy, and the histological report, investigating the type of exposure to asbestos (occupational, neighborhood, household), the survival time since the diagnosis, the latency time, and the relationship to cigarette smoking.

Then, a smaller group was carefully selected, including 40 subjects, subgrouped as follows: ten males who died from mesothelioma and had occupational exposure; ten males who died from mesothelioma and had only neighborhood exposure; ten females who died from mesothelioma and had only neighborhood exposure; and, finally, ten males with occupational exposure who died from asbestosis. For these subgroups, an SEM/EDS analysis was performed on the lung samples collected during the autopsy.<sup>1</sup> The main endpoints consisted in detecting the lung fiber burden, the dimensions, and the mineralogic species of each detected fiber.

The statistical analysis performed on the whole sample showed that there was a significant relationship between the cause of death and the type of exposure. Interesting and significative differences were reported between the different kinds of exposure (occupational, neighborhood, and household) analyzed for the different pathological pictures (mesothelioma, asbestosis, and lung cancer). A significant association was observed between smoke and neighborhood exposure ( $p=0.026$ ). Moreover, among the mesothelioma patients, the survival time was shorter in the subjects with a neighborhood or household exposure. Regarding the SEM/EDS results, interesting differences, in terms of mineralogic species and fiber dimension, were observed between the four groups.

In conclusion, this study provided interesting and novel data about the role of different doses and types of asbestos in causing human pathologies and its interaction with other factors. The present data appear to support the hypothesis that even an exposure to a very small amount of asbestos (due to, for example, a neighborhood exposure) can cause mesothelioma in hypersusceptible subjects.

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#### Asbestos, Scanning Electron Microscope, Mesothelioma



**H63** WIHDRAWN





## H64 A Rare Case of Sudden Death From Hereditary Hemorrhagic Telangiectasia (HHT)

Heather M. O'Connor, DO\*, Medical University of South Carolina, Charleston, SC 29425; Ellen C. Riemer, MD, JD, Medical University of South Carolina, Department of Pathology, Charleston, SC 29425

**Learning Overview:** The goal of this presentation is to report on a rare case of sudden death from HHT.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing features of HHT, since many cases are undiagnosed and may present as sudden death. In previously undiagnosed cases, clinical and/or genetic testing of at-risk family members could be lifesaving.

Reported here is a rare case of sudden death due to HHT in a 66-year-old female with a history of high output heart failure and pulmonary hypertension. At autopsy, multiple Arteriovenous Malformations (AVMs) were throughout the lungs and liver. Additional AVMs were identified on the epicardial surface of the right atrium. Secondary effects of the disease identified at autopsy included cirrhosis of the liver and marked biventricular dilatation of the heart.

HHT is an autosomal dominant disorder with variable penetrance in which agenesis of capillaries results in a direct connection between veins and arteries, known as telangiectasias in mucocutaneous sites and AVMs in visceral organs.<sup>1-4</sup> HHT is associated with multiple gene mutations; the two most common are ENG (endoglin) and ACVRL1/ALK1 (activin receptor like kinase 1), with fewer than 5% of cases having mutations in SMAD4 or GDF2. All of these genes are part of the Transforming Growth Factor Beta (TFG-B) superfamily of regulatory proteins that play various fundamental roles in cellular function, including cell survival, proliferation, and differentiation. Mutations in any of these genes leads to malfunction of this signaling pathway and results in abnormal angiogenesis.<sup>5</sup>

The visceral organs most frequently involved include the lungs, brain, gastrointestinal tract, urinary tract, and liver. Approximately half of all affected individuals have lung involvement, resulting in an anatomical right-to-left shunt, the major consequence of which is impairment of gas exchange leading to local and systemic hypoxemia.<sup>2</sup> Another serious potential consequence of pulmonary AVMs is paradoxical emboli, which can lead to strokes and brain abscesses.<sup>5-6</sup> Although the majority of HHT cases have hepatic involvement, fewer than 10% of these are symptomatic.<sup>3,7</sup> Symptomatic cases often lead to high output heart failure and have a higher risk of portal hypertension, cirrhosis, and encephalopathy.<sup>7</sup>

Those affected by HHT can have a near-normal life expectancy with early initiation of treatment and monitoring.<sup>1</sup> When a case is diagnosed at autopsy, it can lead to identification of at-risk family members who can benefit from appropriate screening and treatment through clinical evaluation and possibly genetic testing.

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### HHT, Arteriovenous Malformations, Endoglin



### H65 Silicosis: Medicolegal Implications of Diagnosis

*Brandon J. Bukovitz, BS\*, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705; Jonathan G. Meiman, MD, Madison, WI; Henry A. Anderson, MD, Wisconsin Department of Health Services, Madison, WI 53703; Erin G. Brooks, MD, University of Wisconsin Hospital and Clinics, Madison, WI 53705*

**Learning Overview:** After attending the presentation, attendees will: (1) be able to recognize and diagnose silicosis at autopsy; and (2) gain a better understanding of the legal ramifications of diagnosis for the patient and the patient's family, including the potential need for expert medical testimony in a court of law.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the diagnostic and death certification accuracy of forensic pathologists, as well as potentially better facilitating compensation for the family of the deceased.

**Introduction:** Despite well-publicized sources of occupational hazard and available means to minimize exposure to harmful particles, silicosis continues to threaten industrial workers in the United States. Although the incidence of hospitalizations and fatalities related to silicosis appear to be declining, recent data suggests that many cases may go unreported. Nationally, Wisconsin has one of the highest incidences of silicosis. Wisconsin has had an occupational disease surveillance program since 1983 and required reporting of outbreaks of occupational disease and toxic substance related diseases, but as of July 1, 2018, reporting statutes were revised to explicitly require reporting of silicosis and several other occupational and environmental diseases. It is hoped this will improve silicosis morbidity/mortality reporting, and detailed investigations can advance understanding of the disease. This study reviews Wisconsin silicosis incidence, compensation, and mortality data—including autopsy results—from the prior 14 years.

**Methods:** A retrospective search of the University of Wisconsin Hospital and Clinics (UWHC) electronic pathology databases was conducted for autopsy cases in which chronic silicosis was diagnosed. Cases were retrieved from the years 2003–2018 utilizing the search term “silicosis.” Additionally, statewide silicosis epidemiologic morbidity/mortality data was provided by the Wisconsin Department of Health Services (W-DHS) for the years 2003–2017, as well as information regarding Wisconsin workman's compensation and employer liability. A literature search utilizing the term “silicosis” and article review was conducted.

**Results:** Between 2003 and 2017, three cases of silicosis were confirmed via UWHC autopsy. All three were men (mean age: 69 years.) Comorbidities included hypertension, coronary artery disease, smoking, and pneumonia. Pertinent histologic findings included collagenous anthracotic nodules, interstitial fibrosis, and refractile foreign bodies. In two cases, silicosis was found to have caused or contributed significantly to death. Overall in Wisconsin, the age-adjusted rate of hospitalizations attributable to silicosis declined between 2003 and 2017 but remains substantially higher than the national average (e.g., 10.1 hospitalizations per million Wisconsin residents in 2010 vs. 1.2 hospitalizations per million residents nationally in 2010, the most recent year in which official reports are comparable). The age-adjusted death rate from silicosis per million residents likewise remains significantly higher in Wisconsin than the national average (i.e., 1.1 deaths per million Wisconsin residents in 2010 vs. 0.4 deaths nationally). A diagnosis of silicosis typically entitles individuals to worker's compensation to cover medical costs associated with their condition. In Wisconsin, the employer may also be required to pay compensatory damages up to \$100,000; this amount may exceed \$100,000 if the employer is found to be non-compliant with Occupational Safety and Health Administration (OSHA) regulations.

**Conclusion:** Historically, Wisconsin has had many foundries, ceramic companies, and industrial complexes where occupational exposure to silica dust is high; this may account for the relatively high silicosis hospitalizations and mortality in Wisconsin as compared to national averages. A diagnosis of silicosis should be carefully weighed by the pathologist in any patient with pulmonary fibrosis and suspicious occupational history, since it carries substantial implications for worker's compensation, compensatory losses, and employer liability.

### Silicosis, Pneumoconiosis, Occupational Lung Disease



### H66 Pulmonary Alveolar Microlithiasis: A Case of Sudden Death

Adrienne E. Sauder, MD\*, Broward County Office of Medical Examiner, Fort Lauderdale, FL 33312; Marlon Osbourne, MD, Broward County Office of Medical Examiner, Fort Lauderdale, FL 33312; Craig T. Mallak, JD, MD, Fort Lauderdale, FL 33312

**Learning Overview:** After attending this presentation, attendees will better understand Pulmonary Alveolar Microlithiasis (PAM), a rare hereditary lung disease, and its histology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing attendees' understanding of PAM and the role of histology in cases of sudden death with non-specific autopsy findings.

**Introduction:** PAM is a rare autosomal recessive disorder characterized by intra-alveolar accumulations of calcium phosphate that eventually impedes gas exchange. It is caused by mutations to the type IIb sodium-phosphate gene cotransporter (SLC34A2) responsible for phosphate homeostasis found on type II pneumocytes.<sup>1</sup> The inability to transport the phosphorus ions out of the alveoli leads to the microlith formation. Most people with PAM have a sibling with the disease. Symptoms usually appear in the third or fourth decade. Patients with PAM are usually asymptomatic in early stages. As the disease progresses, symptoms include dyspnea, dry cough, chest pain, and cor pulmonale. Diagnosis is usually made by imaging or lung biopsy. Chest radiograph would have the "sandstorm" appearance of the diffuse opacifications of both lungs.<sup>2</sup> Histologically, these intra-alveolar accumulations are round to oval concentric laminated microliths. The only effective treatment is a lung transplant.<sup>3</sup>

**Materials and Method:** The decedent was a 32-year-old woman with a history of diabetes and alcohol abuse. She was not taking any medications. She had an episode of vomiting the morning of her death but was feeling better around dinnertime the same day. She was found unresponsive shortly thereafter and was taken to the hospital and was not revived.

**Results:** At autopsy, the lungs were firm and non-compliant. The right lung weighed 1,280 grams and the left lung 1,200 grams. The pleural surface was smooth and shiny. The cut surface was gritty, firm, and consolidated. There was honeycombing of the parenchyma. Microscopic examination of decalcified lung showed lamellated calcium microliths filling most of the alveoli, with no fibrosis or inflammation. All other organs were within normal parameters. Toxicology was performed and was negative.

**Discussion:** This case highlights the importance of histology in correlating non-specific gross findings with microscopic findings. This is an unusual case because the decedent did not know she had PAM, and her death was earlier than most cases reported in the literature. The lack of fibrosis and cor pulmonale findings could indicate a rapid progression, even though it is a disease known for its slow and progressive course. Establishing the diagnosis after death has significance because it allows siblings to be tested and monitored for the disease.

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#### Pulmonary Alveolar Microlithiasis, Sudden Death, Histology

### H67 Tau and Neurofilament Light Proteins in Cerebrospinal Fluid as Biomarkers of the Time of Death

Pierre-Antoine Peyron, MD\*, Département de Médecine Légale, Montpellier 34295, FRANCE; Sylvain Lehmann, PhD, Laboratory of Biochemistry - Clinical Proteomic, Montpellier 34295, FRANCE; Constance Delaby, PhD, Laboratory of Biochemistry - Clinical Proteomic, Montpellier 34295, FRANCE; Eric Baccino, MD, Hôpital Lapeyronie, Montpellier, Cedex 5 34295, FRANCE; Christophe Hirtz, PhD, Laboratory of Biochemistry - Clinical Proteomic, Montpellier 34295, FRANCE

**Learning Overview:** The goal of this presentation is to present an exploratory, cross-sectional study aimed at identifying two new biomarkers of the time of death by means of thanatochemistry.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of how thanatochemistry may help in more precise and reliable estimation of the Postmortem Interval (PMI), thanks to the most recent technological developments in this field.

**Background:** One of the most important and challenging tasks in forensic medicine is the accurate determination of the PMI. Different methods have been proposed, but they all lack precision and reliability. Recent developments in biochemical techniques may provide the opportunity to assist in more precise estimation of the time of death. The study of postmortem chemical changes in closed compartment body fluids such as vitreous humor and Cerebrospinal Fluid (CSF) has shown promise. In the present study, two major proteins of the central nervous system (Tau and Neurofilament Light (NFL) proteins) were investigated in CSF as potential biomarkers of the time of death.

**Objectives:** The main objective of the study was to assess the correlation between the concentrations of Tau and NFL in CSF and the PMI. The secondary objectives were: (1) to determine the inter-individual variability of the concentrations of Tau and NFL for a same PMI; (2) to determine the kinetics of these concentrations over time; and (3) to determine the variability of these concentrations according to the sampling site (lumbar vs. sub-occipital).

**Materials and Methods:** This study was reviewed and approved by the Ethics Committee of the University Hospital of Montpellier, France. Postmortem CSF samples were collected from 100 adult cadavers whose time of death was precisely known, at the mortuary of the University Hospital of Montpellier, France. Individuals with neurological disorders and head trauma were excluded from the study. CSF samples were removed by cisternal and lumbar punctures at different time intervals. Two mL of CSF were obtained at each tap in clean, sterile polypropylene tubes, using a 18G lumbar puncture needle. The cadavers were divided into four groups according to the PMI ( $n=25$  in each group). The samples were taken 0h–6h (group A), 6h–12h (group B), 12h–18h (group C), and 18h–24h (group D) after death. Additionally, CSF samples were collected every 3h from ten cadavers during the first 15h postmortem. All cadavers were kept at room temperature ( $+20^{\circ}\text{C}\pm 2^{\circ}\text{C}$ ) during sample collection. CSF samples were transferred in ice to the laboratory where they were centrifuged for 10min ( $+4^{\circ}\text{C}$ , 1,000g). The clear supernatant was divided into aliquots, then stored at  $-80^{\circ}\text{C}$  until analysis. The rectal and tympanic temperatures at the time of CSF collection were measured using a probe thermometer, and *rigor* and *livor mortis* were assessed.

Concentrations of total Tau and NFL in CSF were measured by conventional and ultrasensitive digital immunoassays, respectively. Total protein concentration was determined using a bicinchoninic acid protein assay.

The correlation coefficient between the concentrations of Tau and NFL in CSF and the PMI was calculated in each case. The inter-individual variability was assessed by measuring the Standard Deviation (SD) of the mean concentrations of Tau and NFL in each group. Linear regression analysis (adjusted for confounders) was used in assessing whether concentrations of Tau and NFL were dependent on the PMI. Paired Student's *t*-test was used to assess the variability of Tau and NFL concentrations depending on the site of CSF collection.

**Results pending.**

**Conclusion:** Thanks to this exploratory study, it will be possible to know if Tau and NFL proteins can be considered as potential CSF biomarkers of the time of death and if further research is needed to confirm these preliminary results.

#### Tau Protein, Neurofilament Light, Biomarkers



### H68 An Uncommon Fatal Ruptured Pseudoaneurysm of the Brachial Artery in an Injected Drug Abuser

Silvia Trotta\*, Bari 70124, ITALY; Antonella Sorrentino, MD, Istituto Medicina Legale, Bari 70124, ITALY; Giuseppe Bertozzi, MD, Department of Forensic Pathology, Foggia 71121, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY; Biagio Solarino, PhD\*, Università degli Studi di Bari, Bari 70125, ITALY

**Learning Overview:** After attending this presentation, attendees will understand a rare case of fatal ruptured pseudoaneurysm of the brachial artery occurring just before heroin self-injection in a chronic drug abuser.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by stressing the importance of scene investigation, autopsy findings, and toxicology results to determine the cause and manner of death in chronic drug abusers.

Intravenous (IV) drug abusers are a population at risk for pseudoaneurysm, an aneurysmal sac with a single layer of fibrous tissue and blood clots present in its wall. In IV drug abusers, this occurs due to traumatic damage by the repeated injections and infections and mostly involves femoral arteries.<sup>1,2</sup>

A 41-year-old man with a previous history of heroin-cocaine abuse presented twice to the hospital emergency room with a painful and swollen arm. The first time, with a clinical suspicion of thrombophlebitis, he refused a surgical consultation and was then discharged with a prescription for anticoagulant therapy. Two days later, he returned to the hospital, and a venous doppler was recommended but the man never did it. Ten days later, he died in his flat in a pool of blood, with an empty syringe and a pre-filled syringe beneath his body; traces of cocaine powder were found in the apartment. At autopsy, there were numerous circular scars on the surface of his arms and legs. A swelling in the right antecubital fossa with a central 3.5cm x 2.6cm defect, exposition of vascular tissue, and clotted blood on the surface was observed. Accurate postmortem examination with a right forearm incision demonstrated a pseudoaneurysm of the right brachial artery with a 1cm x 0.4cm tear on its anterior wall.

Other findings on internal examination included an edematous brain, edematous and anthracotic lungs, and diffuse lymphadenopathy. The section of the pulmonary artery showed scarce blood, as well as in the vena cava; there was no blood in the aorta. Microscopic examination of the vascular wound samples confirmed layers of fibrin-hematic tissue with infiltration of acute inflammatory cells into the surrounding skin specimen. General drug screening by Gas Chromatography/Mass Spectrometry (GC/MS) on the blood sample provided negative results for opiate derivatives, revealing instead the presence of cocaine, opioids, and methadone in the urine sample.

According to medicolegal, histological, and toxicological findings, the cause of death was ruled as hemorrhagic shock due to spontaneous rupture of brachial artery pseudoaneurysm in a chronic drug abuser. Given these results, a direct correlation with acute drug intoxication was excluded. In this presentation, the uncommon role that the intent to self-administer a drug just before vascular rupture could have on the manner of death will be discussed.

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#### Pseudoaneurysm, Brachial Artery, Drug Abuse



### H69 A Fatal Idiopathic Spontaneous Intraperitoneal Hemorrhage (ISIH) (Spontaneous Hemoperitoneum) in a 56-Year-Old Male With Cirrhosis

Gregory M. Dickinson, MD\*, Montefiore Medical Center, Bronx, NY 10467; Hannah C. Jarvis, MRCS\*, Harris County Institute of Forensic Sciences, Houston, TX 77054; Jacob Steinberg, MD, Montefiore Medical Center, Bronx, NY 10467

**Learning Overview:** After attending this presentation, attendees will better understand the signs, symptoms, risks, and findings associated with ISIH.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the difficulties of evaluating a spontaneous intraperitoneal hemorrhage for both clinicians and forensic pathologists and highlight the likely associations to be aware of for improving diagnostic accuracy.

A 56-year-old man with a history of chronic alcohol abuse collapsed at work and was brought to the emergency department. Despite resuscitative efforts, he remained hypotensive and tachycardic. His hemoglobin dropped from 8.9g/dL to 2.1g/dL (reference range 14.0g/dL–17.4g/dL) over the course of several hours. Additionally, the patient's prothrombin time was >200 seconds (reference range: 26.1–33.8 seconds) and his partial thromboplastin time was 24.5 with an International Normalized Ratio (INR) of 2.4 (reference range: 0.9–1.2, respectively). An abdominal Computed Tomography (CT) scan revealed abdominopelvic hemorrhagic ascites without active extravasation. Multiple blood products were transfused, but without any clinical improvement, and the patient died.

At autopsy, the abdomen was distended with a fluid wave. There was 3.5 liters of blood in the peritoneal cavity. Despite a thorough search, the source of the hemorrhage could not be identified. The patient also had hepatomegaly (2,750 grams) with nodular and fatty changes. On histology, the liver showed steatosis and fibrosis, consistent with cirrhosis. The manner of death was natural, and the cause of death was complications of coagulopathy due to severe liver disease.

Peritoneal hemorrhage may be secondary to aneurysmal rupture, solid organ malignancy, inflammatory erosive processes, or trauma. The cause may also be idiopathic.<sup>1</sup> Trauma and non-malignant gynecological conditions account for greater than 90% of peritoneal hemorrhages. The major cause in women is ruptured ectopic pregnancy, and the major cause in men is post-traumatic rupture of the liver or spleen.<sup>2</sup> Non-traumatic peritoneal hemorrhage is a rare condition. ISIH was first reported by Barber in 1909. The term “abdominal apoplexy” was coined by Green and Powers in 1931 as a comparison to its cerebral counterpart of historical significance.<sup>1</sup> ISIH is a diagnosis of exclusion after aortic dissection, ruptured aortic aneurysm, malignancy, gynecologic lesions (ectopic pregnancy), traumatic injury, or other obvious sources of hemorrhage are ruled out. It is thought to be caused by a spontaneous rupture of the smaller abdominal arteries or veins, especially at branch points where small aneurysms may form.<sup>3</sup> Approximately 30% of cases have no identifiable cause.<sup>4</sup> Likely risk factors include arteriosclerosis, essential hypertension, portal hypertension, and liver cirrhosis.<sup>1,4</sup> The exact pathophysiology is unknown but likely represents weakness of the tunica media, which predisposes the vessel to rupture in the face of abrupt increases in pressure. Spontaneous hemorrhage may also be associated with inflammatory and necrotizing processes, such as polyarteritis nodosa and rheumatoid arthritis. Venous rupture is thought to be associated with portal hypertension.<sup>1,3</sup> There is a male predominance, and the majority of cases present between and 50 and 60 years of age.<sup>1,4</sup>

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#### ISIH, Spontaneous Hemoperitoneum, Abdominal Apoplexy



### H70 A Case Report on the Death of a 20-Year-Old Female Due to Complications of Minimally Invasive Transcatheter Atrial Septal Defect (ASD) Closure

Jan Ynav T. Quiz\*, University of Tennessee Health Science Center, Memphis, TN 38163; Marco Ross, MD, West Tennessee Regional Forensic Center, Memphis, TN 38105; Erica Curry, MD, Collierville, TN 38017

**Learning Overview:** After attending this presentation, attendees will better understand: (1) the fatal complication of cardiac erosion in transcatheter closure of ASDs, (2) corresponding risk factors, and (3) possible methods for its prevention.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by raising awareness of sudden death following transcatheter closure of ASD in adults.

ASD is one of the most common congenital heart defects presenting in adulthood. Although small defects may be asymptomatic and not require therapy, closure is usually indicated for larger defects with significant shunts, evidence of volume overload, or in patients at high risk for life-threatening complications.<sup>1</sup> Among obstetric patients, it has been determined that those with unrepaired ASD had a higher risk for pre-eclampsia, small-for-gestational age birth weight, and fetal mortality compared to those with repaired ASDs.<sup>2</sup> The common closure procedures involve open-heart surgery and transcatheter closure. Transcatheter closure, now commonly performed with the AMPLATZER™ Septal Occluder (ASO), was determined to be as effective and yet safer than cardiopulmonary bypass and surgical repair as it avoids their associated risks (e.g., infection); however, this approach does carry a risk for a rare but serious and potentially fatal complication—cardiac erosion.<sup>3,4</sup>

This study describes the case of a 20-year-old woman with a 2.1-cm ASD who underwent transcatheter closure of the ASD postpartum using the ASO and expired two weeks later. Postmortem examination revealed significant hemopericardium due to full-thickness right atrial erosion caused by the rim of the ASO.

A medical literature review indicates several relative risk factors associated with cardiac erosion, mainly deficiency of any rim, device >5mm larger than ASD diameter, and lesser weight:device-size ratio. Median onset of cardiac erosion was determined to be about two weeks.<sup>5</sup> The case described here had a deficient anterior rim, and cardiac erosion occurred two weeks post-procedure. More frequent follow-up echocardiograms may allow for early clinical detection of erosion, which can be successfully repaired with no further complications.<sup>6</sup>

In conclusion, transcatheter closure of ASDs by the ASO is relatively safe and effective compared to open-heart surgical repair. However, cardiac erosion by the ASO is a rare but potentially fatal complication. Early clinical recognition of this complication, possibly using more frequent post-procedure follow-up echocardiography, is essential and can result in successful surgical repair.

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#### ASD, Transcatheter Closure, Cardiac Erosion



## H71 Alcoholic Hepatomegaly: A Case Report and Review of the Literature

Ashley M. Luebbbers, MS\*, Eastern Virginia Medical School, Norfolk, VA 23507; Wendy M. Gunther, MD, OCME, Tidewater District, Norfolk, VA 23510-1046

**Learning Overview:** After attending this presentation, attendees will be familiar with the pathologic features of alcoholic hepatitis and fatal alcoholic liver disease, in the absence of variceal bleeding, and will review the etiology of fulminant jaundice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by assisting in the recognition of fatal and non-fatal features of acute and chronic alcoholism, in particular hepatomegaly and alcoholic hepatitis, in the absence of ascites or esophageal variceal bleeding.

Excessive, prolonged alcohol intake has been shown to cause a variety of hepatic changes, including steatosis, inflammation, and fibrosis; inflammation may result in the development of alcoholic hepatitis in persons with a genetic predisposition.<sup>1</sup> Sequelae include resolution, hepatomegaly, cirrhosis, and/or progression to hepatocellular carcinoma. A host of systemic complications may include hiccups, abdominal pain, splenomegaly with thrombocytopenia, and portal hypertension leading to ascites and esophageal varices. Alcoholic liver damage may be recognized at gross autopsy from hepatomegaly, liver friability due to steatohepatitis, micronodular or macronodular cirrhosis, and jaundice. Although death is commonly due to variceal rupture and gastrointestinal bleeding, it may occur from alcoholic hepatitis alone.

**Case History:** A 36-year-old Caucasian male with a long-standing history of alcohol abuse was found dead in a supine position on the floor next to his bed, surrounded by empty alcohol bottles. Eight months earlier, he had presented to the emergency room with complaints of hiccups and right upper quadrant pain. He denied recent alcohol intake despite an odor of alcohol detected by emergency room personnel; the laboratory reported a blood alcohol concentration of 0.34g/dl. The patient's family stated that he was still drinking heavily on a regular basis. He admitted to consuming upward of 25 ounces (over 700g) of vodka per week. His liver panel results were consistent with liver damage (bilirubin 4.7mg/dL [direct 2.7mg/dL], AST 339 U/L, ALT 76 U/L, alkaline phosphatase 76 U/L) with thrombocytopenia (platelets 65K/uL). Despite impaired liver function, his albumin was normal at 4.1g/dL, and he did not have ascites; his kidney function appeared normal, without evidence for hepatorenal syndrome. An ultrasound examination showed hepatomegaly and fatty infiltration, characteristic indicators of alcoholic liver damage.<sup>2</sup> He was admitted to the hospital for a week with a diagnosis of alcoholic hepatitis, placed on withdrawal precautions, and subsequently discharged with a recommendation to abstain from alcohol. There was no further medical contact before he was found dead in his room.

At autopsy, he showed head-to-foot jaundice, including scleral icterus, and multiple purple contusions consistent with thrombocytopenia. The liver showed profound hepatomegaly (4475g/9.845lbs), about three times the normal weight, and measured 35cm x 26.5cm x 12cm, with both extreme steatosis and micronodular cirrhosis. Aspiration of the gallbladder resulted in 110cc of sludgy bile, although the biliary tree was patent. His spleen, at 582g, was also enlarged to about three times its normal weight. There was no ascites. Esophageal varices were unruptured, with no blood in the gastric pouch. Death was ascribed to fulminant liver failure, due to recurrent alcoholic hepatitis.

**Discussion:** The amount of alcohol intake required to initiate liver failure is approximately 100g-200g per day.<sup>3</sup> In this case, he admitted to an alcohol intake of 700 grams per day. Although this patient was only 36 years old at the time of death (below the average presenting age of 53 years for alcoholic hepatitis), he showed signs of fulminant liver failure superimposed on hepatic cirrhosis and recurrent alcoholic hepatitis. Acute fulminant jaundice may be due to alcoholic hepatitis, hepatotropic viruses, ischemic injury, drugs and toxins (e.g., acetaminophen, anticonvulsants, poisonous mushrooms), and unusual entities such as autoimmune hepatitis. He had no history of exposure to viral agents or hepatotoxic drugs (acetaminophen could not be ruled out). Although he never developed ascites or variceal bleeding, which are common causes of death from alcoholic cirrhosis, his autopsy findings correlated to his presenting symptoms on his prior hospital admission. His fatal liver disease resulted from many of the systemic alterations that have been described in association with alcoholic hepatitis.

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### Alcoholic Hepatomegaly, Alcohol-Induced Liver Failure, Alcoholic Hepatitis





### H72 A Ruptured Infective Aneurysm Presenting as an Acute Subdural Hematoma: A Case of Sudden Death in a Drug Addict With Previously Undiagnosed Bacterial Endocarditis

Michael Alan Hays, MD\*, Tidewater Office of the Chief Medical Examiner, Norfolk, VA 23510

**Learning Overview:** After attending this presentation, attendees will recognize the risk factors for infective aneurysm, familiarize themselves with an unusual presentation of aneurysmal rupture, and recognize causes of non-traumatic subdural hemorrhage.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing clinical suspicion of non-traumatic subdural hemorrhage and suggesting alternate diagnostic modalities beyond routine radiology, where indicated.

Acute Subdural Hematomas (SDHs) are most commonly caused by blunt impact trauma to the head. Non-traumatic (or “spontaneous”) SDH is a rare condition. Known causes of non-traumatic SDH include ruptured cerebral aneurysm. Such cases typically manifest as a combination of subdural and subarachnoid hemorrhage. Presentation of a ruptured aneurysm as an isolated subdural hematoma is exceptionally rare.

Here is reported the case of a 25-year-old female patient who died of a ruptured infective aneurysm that appeared as a pure SDH on clinical imaging. Her medical history included chronic intravenous drug abuse and infection with HIV and hepatitis C. She was discovered unresponsive and nude from the waist down inside a known drug house, was transported to a nearby hospital, and was subsequently transferred to a tertiary referral center. An initial urine drug screen was negative, and no significant traumatic injuries were noted on physical examination. A Computed Tomography (CT) scan of the head revealed a right-sided subdural hematoma measuring up to 1.8cm in thickness, with associated right-to-left midline shift of 1.5cm and herniation of the cingulate gyrus. Emergency craniectomy for evacuation was performed. She subsequently developed a high fever and blood cultures grew positive for Methicillin-Resistant *Staphylococcus aureus* (MRSA). Antibiotic coverage was initiated, and hospital workup revealed endocarditis. She followed a downward course and ultimately died on hospital day seven.

At autopsy, external examination showed a chronically ill-appearing female with no external injuries apart from scattered, small scabs and track marks. Internal examination confirmed the presence of bacterial endocarditis of the mitral valve with associated abscesses of the myocardium, spleen, and kidney. Neuropathological examination of the brain confirmed the presence of subdural hemorrhage and revealed a 2.7cm lesion of the right parieto-occipital cortex, with minimal associated subarachnoid hemorrhage. Microscopic examination of the lesion demonstrated features consistent with a ruptured infective aneurysm. Multiple smaller lesions consistent with septic emboli were identified elsewhere in the cortex, predominantly in the distribution of the right middle cerebral artery. Secondary neuropathological findings included cerebral edema, marked hypoxic-ischemic change, uncal herniation, and infarction of the midbrain.

Non-traumatic SDH is a relatively rare occurrence and has a high risk of mortality. Accurate diagnosis of the underlying cause is critical to effective medical decision making, and possible etiologies include aneurysmal rupture. A recent review of the literature identified no more than eight cases of pure acute SDH due to rupture of a cortical middle cerebral artery aneurysm, as seen in the present case.<sup>1</sup> The case presented here is remarkable in that the circumstances of discovery and initial clinical presentation were suggestive of a non-natural death, including the possibility of a blunt trauma homicide. Patients presenting with acute subdural hemorrhage with no clear etiology necessitate thorough clinical and radiographic evaluation to determine the cause of bleeding.

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#### Infective Aneurysm, Subdural Hematoma, Bacterial Endocarditis



### H73 Sudden Death (SD) in Adults With Congenital Heart Disease (CHD): A Retrospective Review of Cases in the Cook County Medical Examiner's Office

*Lorenzo Gitto, MD\**, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210; *Steven M. White, MD, PhD*, Cook County Medical Examiner's Office, Chicago, IL 60612; *Ponni Arunkumar, MD*, Cook County Medical Examiner's Office, Chicago, IL 60612; *Serenella Serinelli, MD\**, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210

**Learning Overview:** After attending this presentation, attendees will better understand the causes of SD in adults with diagnosed and undiagnosed CHD in Cook County, IL.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information about the anatomic defects (simple or complex, single or multiple) and the medical history in adult CHD cases in a medical examiner's population.

CHD in adults is associated with a high rate of SD, despite improved life expectancy because of better surgical and interventional therapies. Most congenital heart defects are repaired in childhood; however, there may be less severe defects that go undiagnosed, resulting in SD in adulthood. The annual incidence of SD in the entire adult CHD population is relatively low, but because the CHD population is growing and aging, the risk profile for SD may have changed.

The electronic database of the Cook County Medical Examiner's Office in Chicago, IL, was examined using the keyword "congenital," searching for cases of CHD as a primary or contributory cause of death, in the period between July 2008 and July 2018. Cases were reviewed for age, sex, race, cause and manner of death, medical history, type of congenital heart disease, and gross and microscopic cardiac findings. Only subjects between 18 and 99 years of age were included in this study.

A total of 84,820 deaths were reported in the studied period in the Cook County Medical Examiner's Office. Of these, there were 15 cases (0.02%) of adult CHD identified, including 14 natural deaths and 1 accidental death. The age ranged between 21 and 62 years. Seven subjects were Caucasian, six African-American, one Hispanic, and one Asian. The male/female ratio was 2.7/1. A complete autopsy was performed in 14 out of 15 cases with consultation with a cardiac pathologist requested in 7 cases.

Of the 14 hearts examine, 12 hearts were enlarged, 8 were dilated, and 9 showed hypertrophy. Simple cardiac defects were observed in 7 cases (50%): 2 cases with isolated Atrial Septal Defects (ASD); 2 cases with isolated Ventricular Septal Defects (VSD); 1 case with VSD combined with pulmonary stenosis, 1 case of bicuspid aortic valve, and 1 case of congenital anomaly of the left coronary ostium. Complex defects were observed in 7 cases (50%): 2 tetralogies of Fallot, 1 Ebstein's anomaly, 1 double outlet right ventricle, 1 transposition of the great vessels alone, 1 transposition of great vessels combined with tricuspid atresia, and 1 Total Anomalous Pulmonary Venous Return (TAPVR).

Diagnosis of CHD was known in 12 cases out of 15 (80%) and, among these, 11 subjects had undergone prior (single or multiple) surgical repair. In 13 out of 15 cases, CHD was the primary cause of death, while it was a contributory cause in 2 cases. Toxicological analyses were negative in 14 out of 15 cases, while in 1 case the subject was found to be positive for a non-toxic level of ethanol.

Despite the limited number of cases, CHDs were very heterogeneous and SDs were more common in adult males (73%). Almost all the CHDs were previously known. The three unknown CHD cases consisted of valvular diseases or coronary defect, which could explain the absence of clinical signs and, therefore, the lack of diagnoses. It is questionable whether these minor abnormalities can cause SD, but in the absence of other potentially lethal pathology at autopsy, they may be used as a cause of death.

In 11 out of the 12 cases (92%) with a known diagnosis of CHD, surgical correction was performed in childhood and 3 of them required additional surgical interventions in adulthood. Since subjects suffering from these complex CHD may require multiple surgical corrections during their lifetimes, there could be misinterpretation of the original cardiac defect at autopsy because of the distortion of the usual anatomy due to the surgeries. It is important for the forensic pathologist to be trained in recognizing CHDs and the surgical procedures used to treat them. When the diagnosis is not clear, the forensic pathologist should request a consultation with a cardiac pathologist experienced in CHD.

#### Congenital Heart Disease, Adult Cardiac Pathology, Sudden Death



### H74 Strangulated and Incarcerated Internal Hernia: A Rare Complication of Meckel's Diverticulum

*Mark A. Giffen, Jr., DO\*, Jamestown, NC 27282; Lauren E. Dvorscak, MD, New Mexico Office of the Medical Investigator, Albuquerque, NM 87131*

**Learning Overview:** The goal of this presentation is to highlight a rare, potentially fatal complication of Meckel's diverticulum.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how to better recognize potentially fatal complications of Meckel's diverticulum.

Meckel's diverticulum is the most common congenital gastrointestinal abnormality, occurring in between 1%–4% of the population. The diverticulum is a remnant of the embryonic vitelline duct which fails to involute, resulting in a true diverticulum of the small intestine. The embryonic vitelline duct is supplied with blood by the vitelline artery, most commonly supplied from what will become the superior mesenteric artery. This blood supply can also fail to involute or partially involute, resulting in a mesodiverticular band that is most commonly attached to the ileal mesentery.

Most people with Meckel's diverticula are asymptomatic and the diverticulum is found incidentally during another procedure or at autopsy. It is estimated that between 4%–6% of individuals with these diverticula will present with gastrointestinal pathology, most commonly Small Bowel Obstruction (SBO). The causes of SBO vary, but most commonly include volvulus, adhesions, hernias, and intussusception. These complications are more commonly documented in adults and older children.

Meckel's diverticula are difficult to diagnose and their complications typically mimic generic SBO symptoms, including nausea, vomiting, abdominal pain, and abdominal distention, making antemortem diagnosis difficult. If left untreated, they carry a high risk of morbidity and mortality. SBO is even more difficult to diagnose in small children due to the non-specific symptoms and their inability to verbalize their complaints. These complications should be recognized, especially in cases of SD in children with non-specific gastrointestinal symptoms as they can be easily missed during autopsy without careful dissection and a high degree of suspicion.

This presentation will highlight the case of a 17-month-old infant who developed vomiting and decreased oral intake with rapid progression to death within 24 hours of symptom onset. Postmortem, non-contrast computed tomography revealed non-specific intestinal and mesenteric edema with a small amount of free fluid in the abdomen. At the time of autopsy, an internal hernia containing the majority of the small bowel was identified. The hernia contents were contained between the mesentery of a Meckel's diverticulum and a mesodiverticular band attached to the mesentery and retroperitoneal tissues adjacent to the ileocecal valve. The large length of herniated bowel resulted in incarceration with compression of the mesentery and vascular pedicle of the contained small intestine, causing ischemia and strangulation.

#### **Internal Hernia, Meckel's Diverticulum, Mesodiverticular Band**



### H75 Spontaneous Uterine Rupture as a Complication of Placenta Previa and Percreta

*Mark A. Giffen, Jr., DO\*, Jamestown, NC 27282; Lauren E. Dvorscak, MD, New Mexico Office of the Medical Investigator, Albuquerque, NM 87131*

**Learning Overview:** After attending this presentation, attendees will be able to describe abnormal placental implantation and the potential complications that can occur and be able to recognize the findings of uterine rupture on postmortem, non-contrast computed tomography.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting a rare, potentially fatal complication of abnormally invasive placental implantation during pregnancy.

Abnormally invasive placental implantation, also known as placenta accreta, is a rare complication of pregnancy with significant morbidity and mortality. Placenta accreta occurs when the normal decidua basalis fails to form (or only partially forms), and the chorionic villi directly invade into the myometrium. Placenta increta occurs when the chorionic villi invade through at least half the thickness of the myometrium. The most severe form of placenta accreta, placenta percreta, occurs when the chorionic villi invade through the entire thickness of the myometrium. Complications of placenta accreta are life-threatening and may include retained placental components after delivery, severe postpartum hemorrhage, and postpartum infections. Additionally, depending on the invasiveness through the myometrial wall, severe complications such as uterine rupture may occur. Without medical/surgical intervention, uterine rupture as a consequence of placenta accreta may cause severe hemorrhage and rapid fatality.

In addition to placenta accreta, placenta previa is a rare but well-recognized complication in pregnancy. Placenta previa occurs when the placenta implants within the lower uterine segment instead of the uterine fundus or body. This may result in the placenta partially or completely overlying the uterine cervix, which can lead to both maternal and fetal morbidity and mortality.

Myometrial damage is increasing in frequency with Cesarean births, which places women at greater risk for the development of both placenta accreta and previa. Placenta previa and placenta accreta have additional risk factors in common, including scarring from prior surgical intervention or curettage and congenital uterine abnormalities, which often results in them occurring together. Furthermore, the lower uterine segment is prone to placenta accreta because of a thinner endometrial component compared to the uterine fundus or body.

Reported here is the case of a 32-year-old woman who was found collapsed on the floor of her apartment. She was known to abuse both depressant and stimulant drugs and it was suspected that she had succumbed to the toxic effects of illicit substances. No external traumatic injuries were present. Her previous surgical history was significant for two prior Cesarean deliveries. Full body, non-contrast computed tomography revealed an intrauterine, third-trimester pregnancy and a large volume of fluid within the abdomen. It remains unknown if the decedent was aware of the pregnancy or had any prenatal care.

At autopsy, 3.5L of liquid and clotted blood was present within the peritoneal cavity. The anterior and right lateral lower uterine wall was visibly ruptured, with placental tissue extruding through a 6cm defect. An intrauterine, female fetus of a 30-36 weeks gestational age, without gross developmental abnormality, was confirmed. The placenta was implanted in the lower uterine segment and completely covered the internal os of the uterine cervix. Approximately one-quarter of the placental disc had invaded entirely through the myometrium and was covered only by a thin layer of uterine serosa with focal rupture. The area of invasion corresponded with an anterior uterine scar, consistent with prior Cesarean section. Toxicology analysis of postmortem femoral blood samples revealed the presence of methamphetamine, which likely contributed to the development of uterine rupture and massive hemoperitoneum due to increased intravascular pressure adjacent to the thin uterine serosa.

#### **Uterine Rupture, Placenta Percreta, Computed Tomography**



### H76 A Ten-Year Retrospective Study of Risk Factors Associated With Deaths Due to Pulmonary Emboli in Washington, DC

Breanna M. Cuchara, MFS\*, Manassas, VA 20110; Francisco J. Diaz, MD, Washington DC Office of the Medical Examiner, Washington, DC 20024; Sasha Breland, MD, Washington, DC 20024

**Learning Overview:** After attending this presentation, attendees will be acquainted with the risk factors of pulmonary emboli and be familiar with potential new risk factors based upon a ten-year review of pulmonary emboli cases that were investigated at the Washington, DC, Office of the Chief Medical Examiner (OCME).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness and diagnosis of pulmonary emboli based upon the recognition of additional risk factors, therefore potentially decreasing the number of deaths that occur yearly from this preventable disease process.

Pulmonary emboli are thrombi that obstruct the pulmonary arteries and most commonly originate from blood clots that form in the deep veins of the extremities. Common and established risk factors include decreased mobility, pregnancy, obesity, and genetic predisposition. Potential new risk factors will also be looked at, such as drug abuse and decedents who are intellectually delayed. Although detectable and treatable, according to recent CDC data, it is estimated that 60,000–100,000 Americans die each year from pulmonary emboli.<sup>1</sup>

In 2006, the DC OCME investigated 879 natural deaths, of which there were two deaths that were caused by pulmonary emboli.<sup>2</sup> In 2015, four decedents died from a pulmonary embolism out of 477 natural deaths.<sup>3</sup> During this ten-year review, it was found that 43.9% ( $n=40$ ) of the decedents that died from pulmonary emboli were females, while 56% were males ( $n=90$ ). It was discovered that 92% of the decedents were African American and 8% were Caucasian.

During this study, the most common risk factors were examined. Obesity, with the average body mass index of 33.2, was mostly seen in this population. New risk factors were also revealed. It was found that 12% ( $n=11$ ) of the decedents had a history of drug abuse. Crack/cocaine ( $n=5$ ) and alcohol abuse ( $n=4$ ) were most commonly seen among these decedents. Interestingly, there was only one case where drug intoxication was officially listed as a contribution to the decedent's cause of death (acute cocaine intoxication followed by prolonged hospitalization and pulmonary emboli). Therefore, this evidence may or may not support a correlation between pulmonary emboli and drug abuse, but it proves that a certain population may be at risk. Other risk factors that came out of this study were intellectual disabilities ( $n=3$ ), fetal alcohol syndrome ( $n=1$ ), autoimmune diseases ( $n=3$ ), and inability to ambulate ( $n=5$ ).

The OCME has a responsibility to report its data to the public and health care agencies to prevent further deaths from occurring. Although this study resulted in small numbers, new risk factors were revealed. People with drug abuse history or who are intellectually disabled have an increased chance of developing pulmonary emboli. If their intellectual and physical disability prevents ambulation, then their chances will increase further. Analyzing data and promoting discussions about this subject allow agencies to create programs that can reach out to at-risk populations. This research warrants an investigation to ascertain the correlation between drug abuse and the risk for pulmonary emboli.

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#### Pulmonary Emboli, Thrombi, Autoimmune Diseases



### **H77 Determination of Hemoglobin A1c Levels by a High-Performance Liquid Chromatography (HPLC) Method in Bloodstains**

*Kemalettin Acar\**, Pamukkale University, Denizli, TURKEY; *Ayse Kurtulus*, Pamukkale University, Denizli, TURKEY; *Esin Avci*, Pamukkale University Medical School, Denizli 20200, TURKEY; *Volkan Zeybek*, Denizli, Eyalet/yerleske 20070, TURKEY; *Erdi Kutlu*, Pamukkale University Medical School, Denizli 20200, TURKEY; *Süleyman Demir*, Pamukkale University Medical School, Denizli 20200, TURKEY

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**THIS ABSTRACT WAS NOT PRESENTED.**



### H78 A Case of Necrotizing Pneumonia From a Marijuana Water Pipe: A Recreational Experience Gone Wrong

Drake Ryan Thrasher, BS\*, Birmingham, AL 35205; Deanna A. Oleske, MD, District 23 Medical Examiner's Office, St. Augustine, FL 32095

**Learning Overview:** After attending this presentation, attendees will better understand the potential complications that can result from habitual marijuana consumption via a water pipe or “bong” and similarly used devices for inhaled marijuana usage.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing a rare association between rapidly fatal bacterial necrotizing pneumonia and habitual marijuana use with water pipes. This presentation will also add to the knowledge of those conducting medicolegal death investigations and forensic autopsies in cases of sudden deaths in young persons with no medical history and drug history limited to marijuana usage.

A 34-year-old White female with only a known history of chronic marijuana use, was complaining of flu-like symptoms (vomiting, chills, and fever) for two days prior to death. The decedent was found obtunded and in cardiac arrest when Emergency Medical Services (EMS) arrived. The decedent was successfully resuscitated, transported to the hospital, and immediately intubated due to respiratory failure. She presented with multi-organ system failure, lactic acidosis due to prolonged downtime, and a clinical suspicion of Disseminated Intravascular Coagulopathy (DIC). A chest radiograph was performed with comment limited to endotracheal tube position. Limited point of care testing revealed a critical anemic state with markedly decreased hemoglobin levels (5.4g/dl). Influenza A/B screens were negative. No other clinical workup was performed due to the grave prognosis, and the decedent expired approximately four hours post-admittance. Further investigation revealed that the decedent preferred a water pipe or “bong” when using marijuana.

At autopsy, the decedent had a diffuse vesicular rash with petechial hemorrhages over the conjunctivae and chest. Cloudy effusions were in the pleural cavities. The lungs were heavy (lung combined weight: 2,910 grams) and consolidated. The pulmonary parenchyma of both lungs was diffusely necrotic and purulent. Other notable gross findings included an enlarged liver with diffuse steatosis, petechial hemorrhages over the pleural surfaces, epicardial surface, and renal calyces.

Postmortem blood and lung tissue cultures were obtained and submitted. Microbiology results showed growth of an isolated bacterial species (*Streptococcus pneumoniae*) in both submitted lung and blood specimens. Postmortem toxicology testing was significant for common marijuana metabolites, indicating chronic usage.

Histopathological examination of the lungs revealed diffuse areas of dense neutrophilic inflammation with alveolar septal destruction and perivascular infiltration. Additionally, the lungs were diffusely involved with large clusters of pigmented macrophages within the alveolar air spaces and septae. Microscopic examination of the skin from the chest showed sub-epidermal blister with thrombi in the dermal blood vessels. Microthrombi and fragmented red blood cells (schistocytes) were identified in the kidney. The liver showed diffuse hepatosteatosis with thin strands of fibrosis.

The manner of death was natural, and the cause of death was Thrombotic Thrombocytopenic Purpura (TTP) due to Necrotizing Pneumonia due to *Streptococcus pneumoniae* bacteremia, with marijuana abuse listed as a contributing factor.

A review of the medical literature shows few case reports demonstrating a possible correlation between necrotizing pneumonia or related conditions and marijuana usage with a water pipe.<sup>1-3</sup> However, this is the first reported case of *Streptococcus pneumoniae* as the causative bacteria species of necrotizing pneumonia presenting with TTP.

The purpose of this case study is to present evidence to the medical community of potential complications of habitual marijuana usage in the hopes of preventing unfortunate circumstances such as this one, as well as to make the forensic community aware of this unique but known complication to better assist in determining cause and manner of death.

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#### Complications of Marijuana Usage, Necrotizing Pneumonia, Thrombotic Thrombocytopenic Purpura



### H79 Sudden Death in a Case of Hiatal Hernia Mimicking a Bochdalek Hernia

*Vittorio Gatto, MD\*, Sapienza University of Rome, Roma 00185, ITALY; Alessia Quattrocchi, Rome, ITALY; Valentina Fazio, Rome, ITALY; Matteo Scopetti, MD, Sapienza University of Rome, Rome, ITALY; Alessandro Santurro, MD, Sapienza - University of Rome, Rome (RM) 00161, ITALY; Mariantonia Di Sanzo, MD, Rome, ITALY*

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**Learning Overview:** After attending this presentation, attendees will understand the forensic problems related to a fatal case of a giant hiatal hernia in an older patient. Particularly, a complete forensic approach by means of clinical data collection, autopsy, microscopic, and toxicological investigation led to the conclusion that the cause of death was a cardiac compression resulting in electromechanical dissociation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as an example that can help identify a cause of sudden death potentially underdiagnosed. This study provides information regarding a rare occurrence reported through the clinical history and the results of the forensic investigation, providing an in-depth and complete point of view.

A 72-year-old man who had no relationship with his family was found dead in a farmhouse near Rome, Italy. The inspection of the place of death and the external examination of the body by police officers strengthened the hypothesis of a natural death. For this reason, a diagnostic autopsy was performed on the body.

The circumstantial data collected before autopsy, in particular the patient's medical history, suggested that the subject was suffering from a condition of gastroesophageal reflux, but no references to other pathologies have been found. In addition, a toxicological screening test was conducted that gave a negative result.

At the inspection of the thorax, marked emphysema of the right lung was found and the left pleural cavity appeared to be occupied by a voluminous mass that was determined as a subatelectasia of the left lung. Opening of the pericardial sac revealed a condition of adhesive pericarditis. Microbiological samples were also taken, with negative results, and the histological study of the pericardium confirmed the irritative condition.

In examination of the abdominal cavity, the omentum, the transverse colon, the stomach, and the pancreas were absent, with an evident dislocation to the left lobe of the liver. After the mass wall was opened, all the omental apron, the stomach, the transverse colon, and part of the pancreas were detected inside. After a cautious removal of the hernial content, it was evident that the hernial gate was represented by the diaphragmatic hiatus, in absence of congenital alterations of the diaphragm.

This case reports how a giant hiatal hernia that simulates a Bochdalek hernia can lead to sudden death by multiple mechanisms. Compression of the heart can induce the development of cardiac arrhythmias or, as is also shown in this case report, lead to the development of irritative pericarditis. This case demonstrates how a mild pathology, if neglected, can lead to sudden death.

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#### Hiatal Hernia, Sudden Death, Case Report





### H80 Death Due to External Compression of the Trachea in a Case of Multinodular Hemorrhagic Goiter

*Massimiliano dell'Aquila, MD\**, Sapienza University, Department of Anatomy, Rome, Lazio 00169, ITALY; *Alessandra De Matteis*, University Sapienza of Roma, Rome 00100, ITALY; *Alessandro Santurro, MD*, Sapienza - University of Rome, Rome (RM) 00161, ITALY; *Vittorio Gatto, MD*, Sapienza University of Rome, Roma 00185, ITALY; *Matteo Scopetti, MD*, Sapienza University of Rome, Rome, ITALY; *Aniello Maiese*, Sapienza University of Rome, Rome 00185, ITALY

**Learning Overview:** After attending this presentation, attendees will better understand the main mechanisms responsible for external compression of the trachea in a case of intra-thyroid bleeding.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting an extremely rare case of asphyxia due to a massive intra-thyroid hemorrhage with an acute external compression of the trachea.

An 81-year-old White female (142cm height and 45kg weight) with a history of multinodular goiter for about 40 years, following the onset of a clinical condition characterized by acute respiratory failure, was transported to the emergency room by ambulance, where the death was ascertained within a few hours after admission.

A recent cardiologic examination in the clinical records reported, in the absence of risk factors for cardiovascular diseases, a sinus tachycardia with a heart rate of 131bpm (beats per minute), a negative objectivity for signs of cardio-circulatory failures, a blood pressure of 120/80mmHg, and a modest exertional dyspnea. At laboratory analysis, Thyroid-Stimulating Hormone (TSH) 0.01 microUI/mL, FT3 (free triiodothyronine) 4.76pg/mL, and Free Thyroxine (FT4) 2.33ng/mL were found, pointing out a hyperthyroidism pattern, attributable to a Basedow's goiter.

At the autopsy, the thyroid presented a weight of 1,510g and showed a much-increased consistency with an irregular and frankly hemorrhagic appearance, compatible with the development of an extensive hemorrhage within a massive multinodular goiter. An extensive hemorrhagic infiltration of mediastinal soft tissues and a tracheomalacia due to compression and dislocation determined by the bulky thyroid mass were also detected.

The microscopic examination confirmed the presence of a wide hemorrhagic area extended to the perithyroidal soft tissues and showed the presence, in sub-pleural location, of intra-alveolar accumulation of erythrocytes and of amorphous eosinophilic material in the alveolar spaces. Blood congestion in pulmonary vessels was also evident, with diffused atelectasis and emphysema.

The microscopic and macroscopic findings suggested the cause of death was a mechanical asphyxia, not attributable to external agents. The goiter (overly bulky if related with the weight-height ratio of the patient) was complicated by the development of intra-thyroid and intra-capsular multiple hemorrhages, involving perithyroidal tissues and the upper mediastinum, and causing an acute swelling of the gland and an external compression of the trachea, leading to the subject's death.

Conclusively, the case herein described has been never reported in the international scientific literature and offers meaningful insights on the mechanisms underlying death in the event of sudden massive bleeding within the thyroid, resulting in a significant swelling of the gland, with external compression of the trachea and the development of an acute asphyxia.

#### Multinodular Goiter, Intra-Thyroid Hemorrhage, Asphyxia



### H81 Cryptococcal Central Nervous System (CNS) Disease in a Large Urban Forensic Population

Jonee Taylor, MD\*, Office of the Chief Medical Examiner, New York, NY; Rebecca Folkerth, MD, PhD, Office of the Chief Medical Examiner, New York, NY 10016

**Learning Overview:** The goals of this presentation are to: (1) alert attendees to the continued presence of CNS Cryptococcal disease in a forensic population in New York City, a relatively resource-abundant setting during the era of Highly Active Retroviral Therapy (HAART); and (2) provide an overview of organism burden, degree of inflammation, and neuroanatomic localization in CNS Cryptococcal disease, and correlation with the type of immune compromise and other demographic factors.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reminding practitioners that CNS Cryptococcal infection is still noted in a relatively resource-abundant urban forensic setting, even during the era of HAART.

**Background:** Despite the introduction and now widespread availability of HAART, fatal opportunistic infections are still a major problem, especially in the developing world. For example, in 2016, >1 million deaths occurred worldwide from AIDS-related illness, and as many as 15%–20% were attributed to meningitis due to *Cryptococcus* species. In contrast to hospital-based autopsy practice, which focuses on patients already in the health care system (and has limited autopsy volume), forensic practice involves high numbers of unattended deaths, very often among vulnerable or underserved populations. Thus, forensic autopsies, particularly in a large, diverse urban setting, can contribute to a public health surveillance function by highlighting underappreciated trends. The goal of this study was to characterize all cases with autopsy evidence of Cryptococcal CNS infection from the office of the Chief Medical Examiner in the City of New York, during the HAART era.

**Methods:** This study screened its database of cases referred for neuropathology consultation from 2004 to 2014 for decedents diagnosed with Cryptococcal disease and identified 14 cases. (For reference, during this period, 10,916 cases were referred for neuropathology evaluation, of which 313 were decedents with HIV/AIDS.) Microscopic sections included frontal lobe, thalamus, basal ganglia, hippocampus, midbrain, pons, medulla, and cerebellum, stained with hematoxylin and eosin. A variable combination of standard ancillary stains, including methenamine silver, periodic acid-Schiff, and mucicarmine, was used in 11 cases. Neuropathology reports on all, and microscopic slides on 9, were reviewed to semi-quantitatively evaluate: (1) organism burden (scale of 1 [rare or sparse], 2 [moderate numbers], and 3 [numerous]); (2) degree of inflammation (0 [none], 1 [sparse], and 2 [robust, including granuloma formation]); and (3) anatomic location (leptomeninges; perivascular space; and parenchyma). Additionally, demographic features were recorded.

**Results:** Among the 14 cases, 8 were female and 6 male, ranging in age from 20 to 74 years. A majority ( $n=11$ ) were born in the United States, and 2 were born in Haiti; the country of origin for one was unknown. There were 9 Black decedents, 4 Hispanic, and 1 White. Of the 14 cases, 11 carried the diagnosis of HIV/AIDS, and 3 were immune-compromised due to other medical illness. Macroscopically, leptomeningeal opacification was noted in 12, particularly over the cerebellum. Histologically, 12 cases had leptomeningeal involvement, 9 had perivascular, and 9 had parenchymal (most cases had more than one pattern). The organism burden was high in 3, moderate in 7, and sparse in 2. In 2 cases, Cryptococcal meningitis was noted as a premortem diagnosis; however, no inflammation or organisms were seen on microscopy. The degree of inflammation was sparse in 7, and robust in 5, including granuloma formation in 3. The neuroanatomic site most frequently affected was the cerebellum ( $n=8$ ), followed by brainstem ( $n=6$ ), and diencephalon ( $n=5$ ). Of note, one case, with a clinical history of blindness, showed histologic involvement of the optic nerve. Only the non-HIV cases demonstrated granulomatous inflammation, including multinucleated giant cell formation engulfing Cryptococcal organisms.

**Limitations:** The numbers of cases are small and likely reflect selection bias, as not all brains were referred for neuropathology consultation. Sampling bias likely prevented postmortem confirmation of a premortem diagnosis of CNS Cryptococcal disease in a minority of cases.

**Conclusion:** *Cryptococcus*, as an opportunistic fungal infection of the immune-compromised, showed a spectrum of CNS involvement regarding distribution of organisms and host response. Of note, only the HIV-negative immune-compromised individuals were able to mount a granulomatous response. It is further pointed out that, even during the HAART era in our relatively resource-abundant forensic setting, CNS Cryptococcal disease is still a significant finding. If anything, this experience likely underrepresents the true incidence of Cryptococcal disease among the large diverse urban population. This study emphasizes the role of neuropathologic evaluation of immune-compromised decedents for public health surveillance of this fatal complication.

#### HIV, Meningoencephalitis, Opportunistic



### H82 A Review of Shellfish Illnesses and Deaths in Mississippi

Mark M. LeVaughn, MD\*, Mississippi State Medical Examiner's Office, Pearl, MS 39208; Brent Davis, MD, Mississippi State Medical Examiner's Office, Pearl, MS 39208; L.R. Funte, MD, Mississippi State Medical Examiner's Office, Pearl, MS 39208; Anastasia Holobinko, PhD, Mississippi State Medical Examiner's Office, Pearl, MS 39208; Steven A. Symes, PhD, Mississippi State Medical Examiner's Office, Pearl, MS 39208

**Learning Overview:** After attending this presentation, attendees will better understand the epidemiology and spectrum of illnesses and deaths caused by the consumption of, or direct contact with, shellfish from the Gulf Coast region of Mississippi.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the results of clinical reviews and autopsy findings of shellfish disease and death over a period of five years. Risk factors, history of exposure, source and types of organisms, and autopsy findings will be discussed.

The family *Vibrionaceae* consists of eight genera of bacteria, not all of which are pathogenic to humans. Infection caused by pathogenic species of *Vibrionaceae* result in two separate types of illness (i.e., cholera and vibriosis) in humans. Cholera was first reported in the United States in 1832. Of the more than 200 serotypes, only types 01 and 139 are referred to as the cause of cholera. On average, seven cases are reported annually in the United States. Vibriosis is caused by infection with any of the remaining serotypes representing pathogenic species of *Vibrionaceae*. There are approximately 80,000 cases reported annually in the United States with 300 deaths.

Vibrios were recognized as an important pathogen in the 1970s. The Cholera and other Vibrio Illness Surveillance System (COVIS) was initiated in 1988 by the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), and the states of Florida, Alabama, Mississippi, Louisiana, and Texas. Over the past five years, 46 cases of Vibrio infection have been reported in the state of Mississippi by the State Health Department and State Medical Examiner's Office. Fifteen of these cases were caused by *Vibrio vulnificus*; four were fatal.

*Vibrio vulnificus* is a halophilic (salt-requiring), gram-negative, and naturally occurring bacterium commonly found in warm marine environments. It causes disease in humans via consumption of contaminated shellfish or by contamination of an open skin wound by seawater. Ingestion results in vomiting, diarrhea, and abdominal pain. Skin infection results in ulceration, hemorrhagic bullae, and necrosis. In both types of contamination, the infection can progress to bacteremia and result in sepsis and death. Persons with pre-existing medical conditions are 80 times more likely to develop bacteremia. Patients who develop bacteremia have a mortality rate of approximately 50%.

**Case Study:** A 57-year-old male was reportedly stuck by the barbed fin of a salt water catfish while on a fishing boat out in the Gulf. When the decedent reported feeling ill, the Coast Guard was summoned, and the decedent was transported to the hospital. His condition declined; resuscitation efforts were unsuccessful. At autopsy, evidence of systemic epidemolysis was present. Petechiae and purpura were present on the torso and extremities. Based on the decedent's case history, postmortem examination, and microbiology cultures, the death was ruled accidental due to complications of *Vibrio vulnificus*.

#### Vibrionaceae, Vibriosis, Gulf Coast



### H83 A Fatal Dental Extraction: A Case of Ceftriaxone-Induced Anaphylactic Shock in an Inmate

*Massimiliano Esposito, MD, University of Catania, Catania 95123, ITALY; Federico Patanè, MD, Catania 95123, ITALY; Francesco Amico, MD, Medicina Legale, Catania 95123, ITALY; Giulio Di Mizio, MD, PhD, Magna Graecia University, Catanzaro 88100, ITALY; Aldo Liberto, MD, University of Catania, Catania 95123, ITALY; Giuseppe Davide Albano, MD, Foggia 71121, ITALY; Angelo Montana, MD\*, University of Catania, Catania, ITALY*

**Learning Overview:** The goal of this presentation is to underline the role of immunohistochemical technique and to estimate serum concentration of mast cell tryptase as a specific marker to determine the cause of death during suspected anaphylactic shock.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the importance of an accurate anamnesis and to give the patient a detailed explanation of the risks of procedures and the possibility of Ceftriaxone (CTR<sub>X</sub>) anaphylaxis. It is important to conduct an exhaustive immunohistochemical study, with anti-tryptase antibodies and measuring allergen-specific IgE in blood samples from the corpse to indicate sensitivity to certain allergens in order to obtain a reliable postmortem diagnosis.

CTR<sub>X</sub> is a third-generation cephalosporin commonly used for bacterial infections. The incidence of CTR<sub>X</sub>-related hypersensitivity skin reactions is between 1% and 3%, whereas anaphylaxis is rare. Although CTR<sub>X</sub> is a frequently used antibiotic, only a few cases of anaphylaxis in response to the first dose of CTR<sub>X</sub> have been reported. A case of anaphylactic shock after a single dose of CTR<sub>X</sub> without previous exposure to the drug is presented. Signs and symptoms of anaphylaxis are variable and can range from mild skin lesions to fatal reactions. It is generally quite difficult to prevent anaphylaxis, although effective advanced life support and postresuscitation care may improve survival.

The following case concerns a sudden death due to intramuscularly administered CTR<sub>X</sub> after a dental extraction in an inmate. Physicians did not collect an accurate anamnesis and did not offer a detailed explanation about the risks of this drug before injection, about any procedures offered, and precautionary measures before and during the dental extraction.

**Case Report:** A 35-year-old male convict with a medical history of allergic asthma, celiac disease, and known food-induced allergies for fish, fresh milk, peanuts, hazelnuts, walnuts, apples, kiwis, and peaches underwent a dental extraction with intramuscular CTR<sub>X</sub> administration. A few minutes after CTR<sub>X</sub> administration, he suddenly collapsed. Advanced life support with intramuscular adrenaline and resuscitation maneuvers were unsuccessful. Postmortem examination was unremarkable. At the autopsy, gross examination showed mild cerebral edema and white foam in the main bronchi. Lungs were normal in shape, increased in volume and weight, and exhibited small subplurular petechiae. Histological examination revealed polivisceral stasis and mild cerebral edema. Myocardial interstitial edema was also detected. An immunohistochemical technique was used to estimate the mast-cell population using the anti-tryptase antibody as a mast-cell specific marker. A considerable number of degranulating mast cells with extracellular tryptase-positive material were observed. Histological and immunohistochemical (CD4, CD15, CD68, CD20, CD3, CD8, and CD45) investigation of brain specimens revealed a diffuse cerebral edema. Lung samples showed subpleural and interstitial hemorrhage, intra-alveolar and diffuse interstitial edema, and acute stasis. The analysis of other organs was unremarkable. The goal was to detect IgE specific for CTR<sub>X</sub>. A serum concentration of mast cell tryptase from femoral blood was 41.4 µg/l. The laboratory tryptase enzyme immunoassay on blood revealed a tryptase concentration of 136.50 mcg/L (n.v. 0.00–15.00 mcg/L), greater than the cut-off value of 45 µg/l for the diagnosis of anaphylactic shock. The death was attributed to an anaphylactic shock due to CTR<sub>X</sub>, after his very first dose.

#### Ceftriaxone, Anaphylactic Shock, Dental Extraction



### H84 An Intrapericardial Rupture of an Aortic Aneurysm in the Anatomic Aortic Arch Variant: A Multidisciplinary Approach

*Enrica Calabrese, MD\*, University of Ferrara, Ferrara 44121, ITALY; Rosa Maria Gaudio, University of Ferrara, Ferrara 44121, ITALY; Raffaella Marino, MD, University of Ferrara, Ferrara 44121, ITALY; Letizia Alfieri, MD, University of Ferrara, Ferrara 44121, ITALY; Erica Bacchio, MD, University of Ferrara, Ferrara 44121, ITALY; Mauro Coppone, MD, University of Ferrara, Ferrara 44121, ITALY; Paolo Frisoni, MD, University of Ferrara, Ferrara 44121, ITALY; Francesca Gualandi, MD, University of Ferrara, Ferrara 44121, ITALY; Matteo Fabbri, MSc, University of Ferrara, Ferrara 44121, ITALY; Margherita Neri, MD, PhD, University of Ferrara, Ferrara 44100, ITALY*

**Learning Overview:** The goal of this presentation is to present a multidisciplinary approach to a case of an unexpected sudden death due to rupture of an aortic arch aneurysm associated with an atypical aortic arch branching variant incidentally detected during autopsy. To clarify the cause of death, to perform the postmortem genetic analysis and a systematic evaluation of decedent's family members, different professional experts, such as forensic pathologists, clinical geneticists, and general practitioners, were involved.

**Impact on the Forensic Science Community:** Little is known in the literature about non-syndromic familial Thoracic Aortic Aneurysms (TAAs); therefore, this presentation will impact the forensic science community by providing a methodical approach in those cases of sudden/unexpected death occurring in asymptomatic individuals with a postmortem diagnosis of Thoracic Aortic Disease (TAD).

According to the literature, aortic arch variants (i.e., variations in the aortic arch vessel branching pattern) are currently viewed as a marker or risk factor for TAD. Furthermore, about 20% of patients with TAA show a family history of similar disease, indicating an underlying significant genetic component. TAAs are usually asymptomatic until acute dissection or aortic rupture occurs; therefore, they often remain undiagnosed until fatal consequences.

A 53-year-old man, who worked as a carpenter, with a negative past medical history, suddenly collapsed at his workplace due to a cardiac arrest. Cardiopulmonary resuscitation maneuvers were immediately performed but were unsuccessful. He was declared dead by medical personnel 25 minutes after the beginning of the cardiopulmonary resuscitation. Before autopsy, the victim's past medical history, circumstances surrounding the sudden death, and the family's medical history were evaluated in collaboration with the general practitioner. The autopsy, performed two days after death, showed no sign of injury, except for traces of emergency medical care procedures. The most striking macroscopic feature was represented by cardiac tamponade, due to the rupture of a saccular aortic arch aneurysm (7.5cm x 8cm), at the site of a complex atheromatous plaque. It was 3cm in thickness, made of several layers of degenerative material. The autopsy also revealed the presence of an aortic arch variant with a two-vessel branching pattern: the first branch was a common origin of the brachiocephalic trunk and left common carotid artery, and the second one was the left subclavian artery. A histological examination was performed, including microscopic evaluation of the site aneurysm rupture and atheromatous plaque samples.

Since the current aortic arch variants associated with the presence of aortic arch aneurysm could be related to the presence of gene mutations that show an autosomal dominant-codominant inheritance with incomplete penetrance, a genetic evaluation of a cluster of most frequently involved mutations was performed, including FBN1, TGFBR1, TGFBR2, COL3A1, ACTA2, MYH11.

These findings suggest that a methodological approach, which involves collective efforts from general practitioners, forensic pathologists, and clinical geneticists, is essential in cases like this, not only to define the cause of death but also to perform postmortem genetic analysis in order to understand the pathophysiology involved in the genetics of aortic aneurysms and a systematic genetic counselling that could reveal disease-causing mutation and potentially prevent further deaths.

#### Sudden Unexpected Death, Aortic Arch Variants, Thoracic Aortic Aneurysm



### H85 An Interesting Case of a Pregnant Female With Systemic Calcification and Thromboemboli

Tasha Zemrus Greenberg, MD\*, Tarrant County Medical Examiner's Office, Fort Worth, TX 76104-4919; Joshua Smith, BS, Tarrant County Medical Examiner's Office, Fort Worth, TX 76104

**Learning Overview:** After attending this presentation, attendees will gain awareness of an interesting case of systemic calcification in a pregnant female examined at the Tarrant County Medical Examiner's Office.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating medical examiners about possible etiologies of systemic calcification as seen in a pregnant female with no known medical history who died as a result of pulmonary thromboemboli.

On February 3, 2018, a complete autopsy was performed on a 37-year-old White female who was found unresponsive in the bedroom at a private residence. She had no known medical history except for being approximately six months pregnant with recent complaints of feeling ill with cough and vomiting. She had not had any pre-natal care and had only used an unknown walk-in clinic for minor complaints. Reportedly, marijuana pipes were found in the residence, though she had no history of substance abuse.

The autopsy was significant for an intrauterine pregnancy with placenta previa. The fetus was male with no congenital anomalies, consistent with 4–5 months gestational age by weights and measurements. The uterus showed a large fundic leiomyoma, 18cm x 18cm x 15cm, as well as multiple additional smaller leiomyomata throughout the myometrium. There was a 14cm x 10cm x 6cm left ovarian chocolate cyst with a 6cm x 3cm x 2cm lobulated tumor in the wall that corresponded to decidualized tissue on histology. Decidualized tissue was also identified in the left fallopian tube that appeared distorted and markedly thickened grossly.

In the left lower lobe of the lung, there was a large thromboembolus. The kidneys were enlarged and pale, weighing 345gms each. Numerous vascular thrombi in varying stages of organization were noted bilaterally in the hilum and throughout the parenchyma. The right thyroid was nodular, scarred, and calcified. A 5mm right adrenal cortical adenoma was present. There was a 10cm x 7cm x 6cm thin-walled cyst on the right pericardium containing serous fluid. A small posterior left parietal osteoma was present.

On histologic examination, there were diffuse vascular calcifications in multiple organs, including the heart, liver, and spleen. The lungs showed calcification of the bronchial basement membranes, and there was calcification of the mucosal basement membranes in the stomach and pancreas. In the liver, there was calcification of the sinusoidal lining. In the heart, there was also calcification of individual myocytes. The thyroid nodule was consistent with a papillary carcinoma. Multiple additional small organizing thromboemboli were identified on histology throughout the lungs.

Review of the literature identified the differential diagnosis of diffuse vascular calcification to be very wide, including common and rare disorders ranging from premature atherosclerosis, diabetes, parathyroid disorders, chronic kidney disease, uremia, systemic lupus erythematosus, hyperphosphatemia, pseudoxanthoma elasticum, Marfan's syndrome, liver disease, and hyperparathyroidism.<sup>1</sup> The patterns of vascular calcifications vary among these conditions; however, none of them have been reported to have cardiac myocyte or hepatic sinusoid calcifications. A case report of acute fatty liver of pregnancy developing massive intrahepatic calcification indicates that it is usually associated with infectious, vascular, or neoplastic lesions in the liver.<sup>2</sup> In pregnancy, it has been reported to be associated with HELLP syndrome.<sup>2</sup> Cardiac fibroblasts can adopt an osteoblast cell-like fate contributing to heart muscle calcification.<sup>3</sup> No reports of a case with the constellation of findings in this death were found. A similar picture can be seen in idiopathic infantile arterial calcinosis. No reports of a case with the constellation of findings in this death were found.

In summary, this is an interesting case of diffuse systemic calcification of unknown etiology in a pregnant woman who died as a result of pulmonary thromboemboli and had thrombi in the kidneys. The association is uncertain and her death, in the absence of the thromboemboli, certainly could have been attributed to the cardiac myocyte calcification and an arrhythmia.

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#### Systemic Calcification, Pregnancy, Pulmonary Thromboemboli



### H86 Carbohydrates That Kill: Death From Refeeding Syndrome?

*Carolyn E. Lemieux, MD\*, London, ON N5X 3L1, CANADA; Vickie Willoughby, DO, Travis County Medical Examiner's Office, Austin, TX 78787; Keith Pinckard, MD, PhD, Travis County Medical Examiner's Office, Austin, TX 78701*

**Learning Overview:** After attending this presentation, attendees will: (1) be able to define refeeding syndrome, (2) understand the cellular mechanisms and metabolic changes associated with refeeding syndrome, and (3) have developed an awareness of the risk factors and special populations in whom refeeding syndrome may be a factor in causing sudden death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science and forensic pathology communities by raising awareness of refeeding syndrome, a paradoxical differential diagnosis of sudden death in malnourished persons that can present for forensic autopsy. Knowledge of refeeding syndrome in these cases is important for death investigators and forensic pathologists to ascertain a complete clinical history, correlate autopsy features, and order appropriate laboratory testing.

Two cases with possible refeeding syndrome were identified at the Travis County Medical Examiner's Office. A 21-year-old male visited the United States from Mexico. Upon arriving, family was concerned at his emaciated appearance and took him for a large meal. Shortly after eating, he began vomiting and experiencing constipation. His symptoms persisted for 24–36 hours before he was witnessed to collapse and succumb to a presumed illness. At autopsy his Body Mass Index (BMI, measured in  $\text{kg}/\text{m}^2$ ) was  $14.5 \text{ kg}/\text{m}^2$ . His stomach and small and large bowel were empty. Vitreous analysis demonstrated hypernatremia consistent with dehydration and his recent history of emesis. Toxicology was negative for alcohols, illicit and prescription medications. In the second case, a 26-year-old male with a history of anorexia nervosa and prior inpatient and outpatient recovery treatment was found deceased in his residence. At autopsy his BMI was  $14.8 \text{ kg}/\text{m}^2$ . Internal examination demonstrated fluid within the chest cavities and lower extremity edema. Toxicology showed therapeutic level of citalopram. Vitreous analysis demonstrated low levels of sodium; however, the potassium concentration was slightly increased.

Refeeding syndrome is a relatively unknown entity that paradoxically potentiates sudden death in malnourished persons subsequently receiving oral, enteral, or parental nourishment. Earliest descriptions of refeeding syndrome were presented during the last century when emaciated prisoners of war clinically described as having marasmus or kwashiorkor were refeed. Progressive knowledge of anabolic and catabolic states of metabolism, nutritional requirements, and electrolyte physiology has provided an understanding of the cellular mechanisms that underlie refeeding syndrome and supported the development of treatment protocols for gradually increasing nutritional intake.

Currently, however, there are challenges in recognizing refeeding syndrome due to the diversity of predisposing risk factors and populations affected, and variable electrolyte disturbances and their subsequent systemic effects. Predisposing risk factors for malnourishment may include malabsorption, infectious disease, eating disorders, low socioeconomic status, cachexia associated with malignancy, advanced age, and post-surgical patients, particular bariatric surgery patients. Electrolyte shifts may demonstrate combinations of hypophosphatemia, hypokalemia, hypomagnesemia, alterations of glucose, lipid, and thiamine, and changes in sodium and water excretion that can cause dehydration or extracellular fluid compartment expansion and edema. Symptoms leading to possible death may incorporate single or multiple bodily systems, including cardiovascular (arrhythmia, hypotension, heart failure), endocrine (hyperglycemia), gastrointestinal (vomiting, constipation, paralytic ileus, diarrhea), hematological (hemolysis, leukocyte dysfunction), neurological (delirium, seizures), renal (metabolic acidosis or alkalosis, acute tubular necrosis), respiratory (hypoventilation, pulmonary edema, respiratory drive failure), and musculoskeletal (rhabdomyolysis, ataxia, diaphragm weakness, muscle cramps, osteomalacia). Malnourished persons with refeeding syndrome can be evaluated in hospital for the aforementioned symptoms and underlying electrolyte abnormalities with a diagnostic battery of testing. However, such methods and diagnostic certainty are not available postmortem.

#### Refeeding Syndrome, Eating Disorder, Anorexia



### **H87 A Very Affordable Postmortem Computed Tomography (PMCT) Angiography Kit: A Feasibility Study Using an Immersion Pump and 3D-Printed Parts**

*Wolf Schweitzer\**, *Universitat Zurich, Zurich CH-8057, SWITZERLAND*; *Markus Enders, Präparator, Zurich Institute of Forensic Medicine, University, Zurich 8057, SWITZERLAND*; *Dominic Gascho, Universitat Zurich, Zurich CH-8057, SWITZERLAND*; *Michael Thali, MD\**, *Universitat Zurich, Zurich CH-8057, SWITZERLAND*

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**Learning Overview:** After attending the presentation, attendees will better understand that it is not difficult to assemble all parts required for PMCT angiography (PMCTA) using parts from a normal hardware store and from pre-designed 3D models that they can get 3D-printed or print themselves.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a very low cost PMCTA kit (except contrast agent).

**Introduction:** A frequent objection to using PMCTA is the associated extreme costs. In that context, this study has not only identified a small immersion pump to be very affordable, but found it to also achieve better vascular filling than a heart lung machine. Using that pump as the core item, this study developed a PMCTA kit containing all materials needed (with the exception of contrast agent admixture).

**Methods and Materials:** This study developed a parts list made of items available at hardware stores and built the remaining items using 3D design and 3D printing. The kit contains a pump as well as a variable power supply. It contains a silicone tube to attach to a small immersion pump. The tube is fixed to the pump by use of a press-fit cylinder. The tube, at its other end, attaches to a femoral catheter. To make a distinction between the venous and arterial access line easier, red and blue colors may be used for 3D printing. The silicone tube and attached pump are kept in place with a spring clamp and a 3D-printed tube clamp fixture that allows for fast tube slide-in or slide-out. Last but not least, the kit contains two vascular tourniquet sets containing a stylet, an occlusion tube, and a quick-release clamp. These are required to fix the femoral catheter, once in position, to the femoral vessel into which it is inserted. The femoral catheter model appeared to be particularly difficult to design for flawless 3D printing using a standard PLA printer. This study employed Finite Element Analysis (FEM) to examine design issues in relation to repeatedly experienced breaks of earlier models. The kit was tested in an adult PMCTA. Its total cost is around 120 Swiss Francs (CHF) for all devices and parts and 170 CHF if a durable case is included.

**Results:** Vascular filling of the arteries contained scalp arteries; excellent filling also of intracranial arteries and upper extremities past the elbow. Vascular filling of the veins extends also past the Torcular herophili with a full staining of the superior sagittal sinus. Images will be presented.

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#### **Virtopsy, Postmortem CT Angiography, 3D Printing**



### H88 The Detection of Insect Stains From Four Species of Necrophagous Flies on Household Materials Using Immunoassays

David B. Rivers, PhD\*, Loyola University Maryland, Baltimore, MD 21210; Gregory Cavanagh, BS, Loyola University Maryland, Baltimore, MD 21210; Valerie Greisman, BS, Loyola University Maryland, Baltimore, MD 21210; Rebecca Brogan, PhD, Loyola University Maryland, Baltimore, MD 21210; Andrew Schoeffield, PhD, Loyola University Maryland, Baltimore, MD 21210

**Learning Overview:** After attending this presentation, attendees will understand how insect stains can be differentiated from human body fluid stains using a confirmatory immunoassay.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by describing a new method for identification of insect stains found at crime scenes that can be reliably used to distinguish insect contaminants from body fluid evidence.

Any interaction between an insect with a corpse or associated exuded body fluids has the potential to create trace evidence in the form of insect stains. In many instances, the chief culprits producing the contaminates are several species of necrophagous Diptera that feed on a wide range of human tissues. By definition, only one type of insect artifact is officially recognized by bloodstain pattern analysts: insect stains. The Scientific Working Group for Bloodstain Pattern Analysis (SWGSTAIN) has defined insect stains as those bloodstains produced as a result of insect activity.<sup>1</sup> This definition leaves open the possibility of producing insect stains by two methods: insect modification of existing bloodstains or the creation of new stains. It is the latter that is most frequently cited by forensic entomologists, since both regurgitation and fecal elimination can yield insect stains containing human blood. The reality is that necrophagous flies can produce stains or artifacts as a result of feeding on several types of fluids (e.g., blood, saliva, semen, vaginal fluids, decomposition fluids), which yield artifacts that vary widely in terms of shape, color, and size.<sup>2-4</sup> Additionally, deposition of artifacts is not restricted to just foraging adults, as post-feeding larvae and newly emerged adults have the potential to contaminate crime scenes with unique artifacts.

Despite claims that fly artifacts can be detected based on morphological features, alternate lighting, and presumptive chemical tests, few species have been tested by the reported methods for discernment and none have proven to be consistently reliable in distinguishing insect stains from human body stains.<sup>5,6</sup> In an effort to overcome deficiencies in current methods used for identification of insect stains, an immunoassay has been developed that utilizes polyclonal antisera (termed anti-md3) based on a unique cathepsin D-like proteinase found in some cyclorhaphous Diptera.<sup>7</sup> The confirmatory immunoassay (dot blot) recognizes insect stains that contain fly digestive enzyme, specifically fly regurgitate and defecatory or fecal stains.

In this study, artifacts produced by four species of necrophagous flies (*Protophormia terraenovae*, *Calliphora vicina*, *Cynomya cadaverina*, and *Sarcophaga bullata*) were examined using the confirmatory immunoassay to determine if insect stains could be distinguished from a range of human body fluids (e.g., blood, semen, urine, saliva, and feces). Adult flies were fed *ad libitum* human blood, semen, urine, feces, or saliva for 24h at 25°C and permitted to deposit artifacts on a range of household materials: ceramic tile, carpet (plush), t-shirt (cotton), wood block, and unfinished drywall. A lift technique was developed that permitted transfer of fly artifacts from the test materials to filter paper (Whatman #4 110 mm Ø) for dot blot analyses.

Artifact transfers were confirmed visually and with an Alternate Light Source (ALS) using a 450nm emission filter and an orange contrast filter. All species readily deposited artifacts on all test household materials regardless of diet consumed. Despite differences in texture and porosity of the household materials, artifacts of all species transferred to saturated filter paper (Dulbecco's PBS) with apparent equal efficiency based on visual inspection. With all fly species, anti-md3 sera bound to artifacts produced after feeding on semen, blood, feces, urine, and saliva. Binding appeared proportional to the size of the artifact transferred during the lifts. By contrast, none of the human fluids tested positive in the immunoassays nor did lifts from household materials not exposed to flies. There was no evidence of false positives with any of the fly species tested, regardless of diet consumed. Similarly, there was also no indication of false negatives with any of the dot blot assays. However, flies did deposit artifacts not derived from the digestive tract on the test materials that, as expected, did not yield positive reactions with the immunoassay. Such artifacts generally cannot be visually distinguished from regurgitate and defecatory stains and thus can yield results perceived as false negatives.

These observations suggest that immunoassays using anti-md3 sera coupled with a simple lift technique can be used effectively as a confirmatory assay to distinguish fly regurgitate and fecal stains from human body fluids. The new method overcomes the limitations of current techniques and can be performed reliably by anyone properly trained without the need of a forensic expert for consultation.

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### **Insect Artifacts, Immunodetection, Forensic Entomology**



### H89 Diurnal Oviposition Timing by Blow Flies (Diptera: Calliphoridae) in Different-Aged Carrion

Kristi Bugajski, PhD\*, Valparaiso, IN 46383

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the timing of blow fly oviposition in relation to sunrise and how the age of the carrion affects this timing. Species composition and ambient factors will also be presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how the timing of blow fly oviposition in relation to sunrise greatly affects accuracy in estimating the Postmortem Interval (PMI).

Blow flies (Diptera: Calliphoridae) are among the first insects to oviposit (lay eggs) on carrion.<sup>1</sup> The timing of blow fly oviposition is critical for determining a PMI estimation, which is the time that has passed between death and corpse discovery.<sup>2</sup> The objective of this investigation was to gain more information about the timing of blow fly oviposition so that a more accurate PMI can be calculated. Past research in the lab has shown that blow fly oviposition occurs an average of 4.75 hours after sunrise in Northwest Indiana. This research expanded on previous studies by using carrion that had been thawing for different amounts of time to simulate different ages of carrion. To see if there was a difference in the timing of oviposition related to the age of the carrion, three piglets were thawed for approximately 15 hours (“new pigs”), and three piglets were thawed for approximately 55 hours (“old pigs”). The six piglets were then placed in a remote, wooded area one hour after sunrise. The piglets were checked once an hour until oviposition occurred, and it was recorded whether flies and eggs were present each hour. The research was repeated six times in the fall of 2017. Egg masses were collected and reared to the third larval instar stage for identification using taxonomic keys.<sup>3</sup>

The timing of oviposition, in hours after sunrise, was analyzed with respect to temperature, humidity, and light intensity. There was no significant difference found in the timing of oviposition between treatments. ( $t=120$ ,  $df=27$ ,  $P=.441$ ). Oviposition occurred an average of 3.9 hours after sunrise in new and old pigs. The species composition varied by date and between new and old pigs. The most common flies found ovipositing were Diptera: Calliphoridae, *Phormia regina* (Meigen) and Diptera: Calliphoridae, *Lucilia coeruleiviridis* (Macquart). *Calliphora vomitoria* (L.) were only collected on new pigs and were absent on old pigs. *Phormia regina* (Meigen) oviposited on new pigs with the same, or higher, frequency than on old pigs, which is the opposite of what was expected. There were record high temperatures in September 2017 (34°C), and this could account for the slightly early oviposition timing found in this experiment (3.9 hours after sunrise). After three years of research, it was found that the average timing of oviposition in Northwest Indiana is 4.4 hours after sunrise in the fall months. This research has importance in both the scientific and forensic communities, as a more accurate PMI can strengthen the validity of a forensic investigation.

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#### Forensic Entomology, Blow Fly, Oviposition



**H90 WITHDRAWN**



## H91 Transcriptional Markers of Sex Determination for Forensic Entomology

Michelle Jonika\*, Magnolia, TX 77354; Ashleigh M. Faris, PhD, Texas A&M AgriLife Research, Corpus Christi, TX 78406; Carl E. Hjelman, PhD, Texas A&M University Department of Entomology, College Station, TX 77843; Aaron M. Tarone, PhD, Texas A&M University Department of Entomology, College Station, TX 77843

**Learning Overview:** After attending this presentation, attendees will understand how genetic markers can be used to predict the sex of blow fly species of forensic importance, which can aide in determining more accurate Postmortem Interval (PMI) estimates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying genes that can be used for a transcriptional approach to sex identification in blow fly species of forensic importance: *Lucilia sericata* (Diptera: Calliphoridae) (Meigen), *Cochliomyia macellaria* (Diptera: Calliphoridae) (Fabricius), and *Chrysomya rufifacies* (Diptera: Calliphoridae) (Macquart).

Indication of sex when identifying insects from human remains is important for death investigations in forensic science. The age and sex of insects is integral for PMI estimates when assumptions for certain conditions can be met.<sup>1</sup> In forensic entomology, species-specific data is gathered for development rate under different temperature conditions.<sup>2</sup> This data can then be applied to case samples and a minimum PMI can be determined utilizing the known data sets.<sup>1</sup> Within these estimates, there can be error in not considering differences in development times between males and females of blow fly species. Currently, only adult specimens can be identified for sex by sight identification, while immature forms have no standardized method for sex identification.<sup>3</sup> This can lead to inaccuracies in PMI estimates as this difference in development time is not being considered in the estimates. The objective of this research is to create an assay that will address the issue of sex identification in immature blow fly forms that can be applied to forensic casework, yielding more accurate PMI estimates.

Three species, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae), and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) were selected for this study. These species are all prominent within the state of Texas and have varying importance in widespread fields of entomology, such as veterinary entomology for myiasis of sheep, use in medical entomology for wound debridement therapy, and importance in forensic entomology, as they are all commonly found at remains recovery scenes.<sup>3</sup> Developmental differences between males and females within these species can be attributed to sexual dimorphism.<sup>4,5</sup> Within the sex determination pathway, differential splicing plays an important role.<sup>4,5</sup> It occurs during gene expression and will give rise to sex-specific phenotypes.<sup>4,5</sup> For this project, two genes of importance within the sex determination pathway—*transformer* (*tra*) and *doublesex* (*dsx*)—were targeted for analyses.<sup>4,5</sup> For the analyses, whole RNA was extracted and converted to cDNA. Then gradient Polymerase Chain Reaction (PCR) was used to determine the best annealing temperature for the selected primer sets and splicing products were visualized with gel visualization. Products and negative controls were confirmed with quantitative PCR (qPCR).

Currently, three distinct assays have been created that can identify the sex of immature forms within the selected blow fly species. Using accepted methodology within the field, these assays were created to target the *tra* sequence in *C. macellaria* and *L. sericata* and the *dsx* sequence in *C. rufifacies*. In addition to the assays created, an identification and error rate for this assay were determined to align with the need for known error rates within the field of forensic science. The assays will help advance forensic entomology by allowing for more accurate postmortem interval estimates in death investigations.

The application of this work can be seen in other projects as well. Currently, there is known gene expression data for *L. sericata*, as well as in progress gene expression data for *C. macellaria* and *C. rufifacies*.<sup>6</sup> With the help of this assay, we can determine whether gene expression has bias in the sexes and the amount of expression each sex may see. This assay can also be useful outside of forensic use in veterinary and ecological applications, such as for sex-specific biological control and better understanding sex-specific traits that may arise in species.<sup>7</sup> With this assay, not only will the field of forensic entomology benefit, but many other fields of entomology as well.<sup>7-9</sup>

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### Forensic, Entomology, Genetics



### H92 An Investigation of Volatile Profiles of Specific Human Organs During Decomposition

Lena M. Dubois, MSc\*, University of Liège, Liège 4000, BELGIUM; Pierre-Hugues Stefanuto, PhD, University of Liège, Liège 4000, BELGIUM; Katelynn A. Perrault, PhD, Chaminade University of Honolulu, Honolulu, HI 96816; Jean-François M. Focant, PhD, University of Liège, Liège 4000, BELGIUM

**Learning Overview:** After attending this presentation, attendees will understand which Volatile Organic Compounds (VOCs) are released from specific human organs, as well as their trends over time as organs decompose.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the potential of decomposition VOC profiling for postmortem examinations and its value as a tool to improve postmortem interval estimation and/or the determination of antemortem conditions.

The human body is made up of multiple complex biological systems. Despite great advances in science and technology, understanding how these systems are linked together and their influence on each other remains a complex task. Death involves the shutdown of these systems, initiating a sequence of events that breaks the body down into its smallest components; these processes are influenced by intrinsic factors such as diet, age, or gender and extrinsic factors such as temperature or humidity. Comprehensive knowledge of human decomposition processes would allow advancements to be made in many crucial forensic applications, including the determination of the postmortem interval or the examination of physical conditions prior to death, which can help to reconstruct antemortem events.

Comprehensive Gas Chromatography coupled to Time-Of-Flight/Mass Spectrometry (GCxGC-TOF/MS) is commonly used to study complex mixtures of VOCs from biological specimens. The objective of this research was to determine the VOCs present during the decomposition of human tissues, along with their respective postmortem trends over time using GCxGC-TOF/MS. Previous research has been largely focused on the analysis of VOCs produced by whole animal or human cadavers. However, this study aimed to identify whether the VOCs produced as a result of decomposition differ between specific organs within a body and, further, to determine the extent of variation between organs in different individuals. The study design allowed for intra- and inter-cadaver comparison for each organ sampled. Heart, lung, liver, kidney, and blood were collected from five bodies. The headspace was monitored during the decomposition process. Tissues were split into replicate glass jars and the headspace was sampled by dynamic pumping onto sorbent tubes that were further thermally desorbed onto a GCxGC-TOF/MS system. Due to the number of cadavers, organs, replicates, and time points analyzed, a large amount of data was obtained, leading to challenges in the integration, interpretation, and representation of the results. To monitor the temporal changes in VOC profiles, multivariate statistical methods, such as Principal Components Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were applied to the dataset to evaluate trends and differences in subgroups.

The first approach was an intra-cadaver approach, where the profile from each organ was compared within a single body for all measured time points. The second approach consisted of an inter-cadaver comparison, where the VOC profiles for a single organ were compared between different bodies at each time point. The intra-cadaver analyses demonstrated that all organs from one body followed a similar trend, despite distinct differences at each time point. Examining the temporal trends, some cadavers exhibited similar trends; however, it was challenging to determine global trends across samples from all bodies. The inter-cadaver analysis focused on the comparison of the different tissues types from the five bodies at selected time points. Distinctive VOCs for each tissue for each time point could be determined. Furthermore, differences were demonstrated for the compounds produced from each organ, which is likely related to their composition based on the different functions they perform within the body. Differences observed in the VOC profiles between the analyzed bodies could range from the individual diet and lifestyle to the cause of death, as all these aspects have an impact on taphonomic processes.

This research is significant because it is the first study monitoring a high number of human tissues during decomposition. The increased number of samples and the use of technical replicates allowed a high quality of analytical data and an assessment of variance for inter- and intra-cadaveric samples. This will provide a foundational basis for the future implementation of VOC profiling from cadavers in forensic casework.

#### Decomposition, Volatile Organic Compounds (VOCs), Human Organs



### H93 An Investigation of the Decomposition Odor Profile Produced by Postmortem Microbes

Terezie Cernosek, Chaminade University of Honolulu, Honolulu, HI 96816; Kevin E. Eckert, MS, Honolulu Police Department, Honolulu, HI 96813; David O. Carter, PhD, Chaminade University of Honolulu, Honolulu, HI 96816; Katelynn A. Perrault, PhD\*, Chaminade University of Honolulu, Honolulu, HI 96816

**Learning Overview:** After attending this presentation, attendees will understand the importance of building a reference library of Volatile Organic Compounds (VOCs) produced by postmortem microbes, as well as the general trends associated with VOC evolution from three postmortem microbial species.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the correlation between results of VOC profiling and the postmortem microbial clock, which may improve postmortem interval estimation and/or search and recovery procedures.

VOCs are the category of decomposition byproducts that comprise the odor associated with decomposing remains. While there have been substantial leaps forward in recent years toward characterizing this odor under various conditions (e.g., postmortem interval, geographical region, season), there exists a large gap in knowledge of the underlying mechanisms that produce these VOCs. A decomposing body is a complex resource of biotic and abiotic components, meaning that volatile by-products cannot always have their exact sources established. Substantial information has become available regarding the microbial community that exists on decomposing bodies, which may provide insight into the sources of critical VOCs released throughout decomposition. As VOCs are now being more closely investigated with hopes of improving cadaver-detection dog practices and introducing complementary portable sensor approaches, it is increasingly important to understand the relation of VOC production in relation to the postmortem microbial community.

The purpose of this study was to investigate VOC emissions from bacteria collected from decomposing remains using Solid-Phase Microextraction Arrow combined with Gas Chromatography/Mass Spectrometry (SPME Arrow-GC/MS). Three postmortem bacterial species (*Bacillus subtilis*, *Ignatzschineria indica*, and *I. urei*) were cultured in headspace vials on standard nutrient agar at 24°C and monitored over a period of five days. The hypothesis was that these species would release VOCs that have been previously identified in decomposition odor in temporal trends throughout their growth cycle.

The results of this work indicated that each species monitored in this study exhibits a specific VOC profile. In addition, VOCs monitored over time exhibited temporal trends; some compounds trended upward in abundance whereas others trended downward, depending on the compound and species of bacteria. SPME Arrow was effective and reproducible in producing bacterial VOC profiles. *I. urei* was the only major producer of dimethyl disulfide, a key decomposition VOC previously identified in all decomposition odor studies. Other compounds of interest included various alcohols, ketones, aldehydes, and aromatics.

This research is significant because it is the first primary study that links decomposition odor with postmortem microbial community. The trends developed in this study will assist in developing more accurate portable sensor arrays for decomposition odor, as well as understanding the dynamics and variability of VOC production in previous literature. Future work on additional postmortem bacterial species and with more advanced chromatographic technology will assist in building a VOC database that can be accessed in future approaches profiling VOCs from whole cadaver decomposition in postmortem examination scenarios.

#### Taphonomy, Volatile Organic Compounds (VOCs), Postmortem Microbial Community



### H94 Estimating Postmortem Interval (PMI) Using Microbial Succession in Human Cadaver Rib Bones

Heather L. Deel\*, Fort Collins, CO 80525; Aerial D. Belk, MS, Colorado State University, Fort Collins, CO 80525; Aaron M. Lynne, PhD, Huntsville, TX 77341; Sibyl R. Bucheli, PhD, Sam Houston State University, Huntsville, TX 77340; Zech Xu Zhenjiang, MS, University of California San Diego, La Jolla, CA 92093; Franklin E. Damann, PhD, DPAA CIL, Offutt Air Force Base, NE 68113; David O. Carter, PhD, Chaminade University of Honolulu, Honolulu, HI 96816; Rob Knight, PhD, University of California San Diego, La Jolla, CA 92093-0763; Jessica L. Metcalf, PhD, Colorado State University, Fort Collins, CO 80523-1171

**Learning Overview:** After attending this presentation, attendees will understand microbial decomposition of human cadavers, how microbiome technologies can be used to estimate the PMI, and how microbial invasion into human rib bones during decomposition may be developed into a novel form of physical evidence for estimating PMI.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of novel microbiome tools that may be useful in investigations of unattended death scenes, in which PMI is unknown.

In crime scene investigations, estimating the PMI is critical for validating alibis and, in some cases, identifying the deceased. Although there are multiple methods to estimate PMI, there are few tools that are accurate after the first two weeks of decomposition. Therefore, a new tool for estimating PMI on the scale of weeks to months is needed. Microbes are major drivers of decomposition that could serve as evidence of microbial succession in a similar way across bodies during decomposition. Previous research has demonstrated that microbiome data collected from both mouse models and human bodies can be used to create an accurate “microbial clock of death.” This study hypothesized that the invasion of microbes into bone is likely a slow process and tracking the succession of microbes into bones after death may provide accurate estimates of PMI for longer timeframes of decomposition.

To test this hypothesis, rib bones from six human bodies were collected, beginning at approximately three months after death. For each body, one rib was collected every three weeks for a total of 48 sampled ribs. Research was conducted at the Southeast Texas Applied Forensic Science Facility, an anthropological research center in Huntsville, TX. After sample collection, samples were shipped to Colorado State University, where each of the rib samples were processed by excising a portion of the bone with a Dremel® drill, cleaning the newly excised piece with bleach and Ultraviolet (UV) irradiation, then pulverizing it into a fine powder. Each of the bone powders were demineralized, then DNA was extracted using the DNEasy® Powersoil Isolation Kit following the Earth Microbiome Project standard protocols. The bacterial microbiome in each sample was then characterized using 16S ribosomal RNA (rRNA) amplicon sequencing on the Illumina® MiSeq® platform at the University of California, San Diego. Data analysis using Quantitative Insights Into Microbial Ecology revealed that similar microbes invade the rib bones during decomposition. This study discovered that PMI could be estimated within approximately one month over a decomposition period of nine months by constructing a random forest regression model using 16S rRNA data. These results are promising because the error of the model (~4 weeks) cannot be more accurate than the sampling frequency (~3 weeks). Therefore, this initial study demonstrates that utilizing the invasion of microbes into bone may be useful for estimating PMI on a longer timescale, and the model may be improved with studies that include more frequent sampling.

#### Bone, Microbiome, Postmortem Interval





### H95 Investigating Italian Criminal Cases' Postmortem Microbiome Signatures

Gulnaz T. Javan, PhD\*, Alabama State University, Montgomery, AL 36104; Holly L. Lutz, University of Chicago, Chicago, IL 60637-1508; Sheree J. Finley, PhD, Alabama State University, Montgomery, AL 36104; Neil Gottel, BSc, University of Chicago, Chicago, IL 60637; Silvia D. Visona, MD, University of Pavia, Pavia 21100, ITALY; Antonio M.M. Osculati, MD, University of Pavia, Pavia 27100, ITALY; Jack A. Gilbert, PhD, University of Chicago, Chicago, IL

**Learning Overview:** After attending this presentation, attendees will understand how to use postmortem microbial diversity of reproductive organs, spleen, etc. from Italian cadavers with times of death up to 15 days to aid in the correlation of time and manner of death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing statistically significant next generation sequencing data that may realistically support crime scene investigation findings (homicide, suicide, etc.).

There is active microbial life in the human body after death throughout the stages of human decomposition, and reproductive organs are among the last internal organs to deteriorate. The role that microbes play during decomposition may provide critical data that is potentially useful for the determination of the time or manner of death. Previous thanatomicrobiome studies using corpses from the United States and Finland established statistically significant differences in microbial signatures between two geographical locations. However, the impact of microorganisms on human decomposition in Italian corpses has yet to be fully elucidated for death investigations. In the present study, 16S ribosomal RNA (rRNA) gene amplicon sequencing of DNA extracts was performed using 40 Italian cadavers' internal organs. The samples were obtained from the University of Pavia's Department of Public Health, Experimental and Forensic Medicine. The tissues were collected from between 1-5 internal body sites, namely brain, heart, liver, spleen, and reproductive organs. It was hypothesized that the microbiome profiles associated with decaying reproductive organs maintain signatures that are indicative of the time and manner of death. This study tested the hypothesis by surveying Italian cadavers with postmortem intervals between 24 hours and 15 days with causes of death of homicide, suicide, natural, and accident. All cases were Caucasian with the exception of two, who were of South American nationality. The ages of the cases at the time of death ranged from 16 to 89 years old.

Results of these studies showed that 16S rRNA gene amplicons demonstrated alpha diversity of microbial communities and were significantly different between organ types. For example, the alpha diversity of prostate- and uterus-associated microbial communities was significantly greater than ( $p < 0.005$ ; Kruskal-Wallis) heart and liver. Microbial beta diversity was weakly, but significantly, correlated ( $p < 0.001$ ; ADONIS) between gender ( $R^2=0.031$ ), Body Mass Index (BMI) ( $R^2=0.053$ ), manner of death ( $R^2=0.073$ ), and postmortem interval ( $R^2=0.04$ ). Spleen, liver, heart, and brain microbial diversities were significantly enriched in the cyanobacterial class 4C0d-2. Furthermore, prostate and uterus samples were depauperate for this class, but were dominated by *Bacilli*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Clostridia*.

In conclusion, the postmortem microbial diversity in cadaver organ tissues was analyzed by high-throughput next generation, 16S rRNA amplicon sequencing techniques. This rapidly developing technique is likely to provide several discoveries in DNA casework. This study confirmed that the alpha and beta diversities varied significantly among internal organ tissues. The significant enrichment of specific bacteria show correlations to several factors, particularly gender, BMI, and postmortem interval, which may provide insight into several aspects of medicolegal death investigations. This data will be used to build statistical models to generate a predictive environment whereby the microbiome of these internal tissue samples can be used to predict the time and manner of death for a given corpse.

#### Cadaver Internal Organs, 16S rRNA, Thanatomicrobiome



### H96 The Application of Eukaryotic Community Succession on Porcine Remains for Postmortem Interval (PMI) Estimation

Luisa Forger, MS\*, Dumfries, VA 22026; Michael Shane Woolf, MS, Charlottesville, VA 22903; Tal Simmons, PhD, Virginia Commonwealth University, Richmond, VA 23284; Jenise Swall, PhD, Virginia Commonwealth University, Richmond, VA 23284; Catherine Cupples Connon, PhD, Virginia Commonwealth University, Richmond, VA 23284; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA 23284

**Learning Overview:** After attending this presentation, attendees will understand how changes in the structure of eukaryotic communities found on decomposing remains may aid in the estimation of PMI.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting a novel area of research that uses eukaryotic communities to provide a useful alternative to traditional PMI estimation techniques by employing next generation sequencing and statistical modeling. Furthermore, this study explores the use of eukaryotic communities for such estimations, an underresearched area.

Determining time since death is an essential component of forensic investigations to complete victim timelines, eliminate suspects, and corroborate the testimonies of those involved. Current techniques for estimating PMI involve observing the physical appearance of remains against known time-/temperature-related changes to calculate a time frame. Recent studies on porcine, murine, and human models have shown that the succession of the microbial community of remains can be used as an alternative method to estimate time since death.<sup>1-5</sup> However, these studies had insufficient replicates, were performed in a laboratory setting, or could not provide long-term estimations. The goal of this study was to combat these issues by performing a better-replicated ( $N=6$ ) field study on a porcine model over an extended period (two months, or 1,703 Accumulated Degree Days (ADD)) and to characterize the eukaryote community of decomposing remains, a topic that is underresearched.

Skin microbial samples were collected from the torso of each set of remains every day during the first week, on alternate days during the second week, and once a week for the remainder of the 60-day period. The eukaryote community of each sample was determined using 18S recombinant DNA (rDNA) MiSeq<sup>®</sup> sequencing. Sequence data were analyzed in the mothur pipeline (v1.39.5) and subsequent statistical analyses were performed in R (v3.4.3). The relative abundance of eukaryote taxa across time points and an Analysis of Molecular Variance (AMOVA) indicated similarities between sequential ADD, but significant differences in the eukaryote communities as different stages of decay were reached. At Level 5 (Family), fresh remains (0–57 ADD) were characterized by the combined presence of *Trichostomatia* (12.1%), *Saccharomycetaceae* (5.6%), *Rhabditida* (18.9%), and *Trichosporonaceae* (11.0%). During bloat and active decay (87–209 ADD), *Diptera* (86.4%) was the most abundant family. At the advanced decay stage (267–448 ADD), *Rhabditida* (34.3%) was predominant, but *Coleoptera* (19.3%) also appeared toward the end of this stage. Dry/skeletal remains (734–1,703 ADD) were dominated by the combined presence of *Dipodascaceae* (33.3%), *Trichosporonaceae* (18.9%), *Debaryomycetaceae* (32.3%), and *Sporidiobolaceae* (8.8%). A random forest model generated using Level 5 taxa for the first 15 days of decomposition (0–448 ADD) explained 84.65% of the variability observed with a Root Mean Square Error (RMSE) of 58.6 ADD (approximately 2 days). Another model generated using Level 5 taxa from all collection times explained 89.51% of the variability observed with an RMSE of 178.1 ADD (approximately 6.6 days). Both models show improvement over those generated using only bacterial community succession data and highlight the importance of the microbial eukaryote community throughout the process of decomposition.

In conclusion, this study will impact the attendees by increasing their understanding of methodologies currently in place and their awareness of emerging forensic techniques for PMI estimation. It will also provide recommendations on how investigators may better approach crime scenes to preserve and collect evidence for use in necrobiome sequencing. Finally, the presentation will highlight how the application of next generation sequencing may change the way that postmortem interval is determined by providing a supplemental technique to traditional estimation methods.

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#### Postmortem Interval, Next Generation Sequencing, Eukaryote



### H97 iFly: Mobile Software for Recording Data From a Carrion Source or Death Scene

*Trevor I. Stamper, PhD\*, Purdue University, West Lafayette, IN 47907*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the capabilities of iFly, a new mobile application for recording data from carrion or death scenes, with a focus on forensic entomological specimen collection and Total Body Score (TBS) estimation. Decomposition scenes rich in entomological material present numerous data recording and preservation challenges. Because of this, most entomologists construct their own “scene data sheets” to record what they consider to be necessary data. iFly was created to provide a flexible digital platform for recording this data from the scene and for sharing data between users in a standard format.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how a mobile application for forensic entomological and TBS data collection can assist in research projects or cases.

iFly integrates a customizable scene documentation system, device enabled “hidden” photography, remote weather station data acquisition, a proof-of-concept built-in visual key system, and precise annotation of sampling location for multiple types of instrumentation (temperature, humidity, wind speed, light level, invertebrate samplings, etc.). Three standard models—domestic pig, human male, and human female—come pre-built into iFly. These standard models allow for concise annotations that reflect the underlying anatomical body locations unique to each model. Tagging samples to the underlying anatomical position on the body allows for the automated acquisition of body location along with specimen or feature documentation—creating the potential to move forensic entomology further into the “big data” future of data analysis. To facilitate this, a MATLAB® utility was created to allow for the customized creation of new body outlines (which would then need to be curated by the iFly team). iFly allows the user to create their own environmental parameters for data sampling and allows the user to compute decedent TBS from the in-application evaluation of photo evidence, as well as the opportunity to identify some entomological specimens using a proof-of-concept built-in visual key.

Cases can be organized around any number of single- or mixed-model assemblages using the default models in iFly. Each model can then be tracked through any number of sampling events. This allows for complicated research set-ups, multiple victim events, or the precise collection of data for carrion ecology student projects at user-defined time increments. Scene data collection layouts can be formatted as a template to conform to pre-established standard operating procedures, allowing for many users to record the same data. iFly allows for the first-ever routine data exchange between carrion ecology or vertebrate decomposition researchers. Individual screens can be exported as .csv files, or the entire case can be exported for peer-to-peer transfer of cases on iFly enabled devices. Photographs imported or taken within iFly are protected from the normal iPad® camera roll, meaning they cannot be accessed from any other app on the device.

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**Mobile Application, Total Body Score, Forensic Entomology**



## H98 International Associations of the Postmortem Microbiome

Jennifer L. Pechal, PhD\*, Michigan State University, East Lansing, MI 48824; Carl J. Schmidt, MD, Wayne County Medical Examiner's Office, University of Michigan, Detroit, MI 48207; Heather R. Jordan, PhD, Mississippi State University, Mississippi State, MS 39762; M. Eric Benbow, PhD, Michigan State University, East Lansing, MI 48824

**Learning Overview:** After attending this presentation, attendees will better understand which metadata has the most impact on structuring a human postmortem microbiome dataset from a multi-country study.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight as to how regularly collected case metadata, such as sex, age, body mass index, manner of death, or country, influenced the human postmortem microbiome.

The use of high-throughput sequencing and advanced statistical modeling to characterize the postmortem microbiome during death investigations has experienced a recent surge. This presentation will describe the human microbiome associations from samples collected during routine death investigations across four countries.

Samples were collected during routine death investigations from four medical examiner's offices (or the international equivalent) located in: Salzburg, Austria; Lille, France; Naples Italy; and Wayne County, Detroit, MI, United States. The individuality of the death circumstances and death investigation protocols within each country is key to acquiring a broad survey of samples from non-targeted case demographic or circumstances of death.

Microbial samples were collected in 2014–2017 from a total of 242 cases. Each case needed to meet the following criteria for inclusion in this dataset: (1) adult ( $\geq 18$  years old), and (2) known circumstances of death. To collect the microbial communities, sterile DNA-free cotton-tipped swabs were individually rubbed against the external surfaces of three anatomic locations (mouth, nose, and rectum) as part of the office's established death investigation protocol. Individual swabs were extracted under aseptic conditions using a commercially available kit with a modified protocol, and the resulting DNA was quantified using commercially available kits for a fluorometer and a microchannel-based automated electrophoresis system. Targeted amplicon (16S ribosomal RNA (rRNA) V4 gene region) high-throughput sequencing was performed on individual sample libraries using a 2x250 base pair, paired-end approach. Samples were processed using an open-source bioinformatic pipeline and statistical analyses, including machine learning algorithms, to identify significant associations among the targeted gene amplicon sequences (microbiome) and case metadata from four countries located in North America and Europe.

Microbial analyses results confirmed distinct postmortem microbiome signatures based on anatomic location and estimated postmortem interval, as previously shown.<sup>1</sup> Alpha-diversity differences (e.g., observed species, taxon diversity) were distinguished among anatomic location postmortem microbial communities. Visualization of these differences were clearly seen in principal coordinate analyses based on phylogenetic similarity (abundance weighted UniFrac distances), demonstrating strong intra-anatomical location similarity. Samples were partitioned based on these anatomic location differences for subsequent analyses. Supervised machine learning analyses classified metadata sources based on sequence variant community composition. Bacterial species within Firmicutes were the most important features for all metadata tested structuring this dataset.

In conclusion, this dataset further highlights the spatial and temporal variability of the human postmortem microbiome across an international, large-scale survey of death investigation. Potential pan-microbial signatures of forensic importance, including ubiquitous bacterial species, were identified within this dataset. There is a tremendous need for pathologists and researchers to continue to work together to identify robust microbial signatures that could be routinely used to inform questions of interest during death investigation across the globe. Additional sample collection, processing, and modeling is imperative to validate the models developed from individual datasets, such as this one, to confirm or identify additional postmortem microbial biomarkers.

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**Forensic Science, Postmortem Microbiome, Forensic Pathology**



### H99 The Composition and Shifts of Soil Bacterial and Fungal Communities Following Cadaver (*Sus Scrofa* Domesticus) and Plant Litter (*Quercus Robur*) Burial

Chawki Bisker, MSc\*, Teesside University, Middlesbrough, North Yorkshire TS1 4PS, UNITED KINGDOM

**Learning Overview:** After attending this presentation, attendees will understand how soil bacterial and fungal communities change during carcass decomposition and the potential exploitation of this phenomenon to estimate time since death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an insight into the shifts in soil bacterial and fungal communities up to 24 months after piglets' burial in contrast with oak leaves decomposition. Furthermore, it suggests the use of soil bacterial and fungal communities to assess time since death and locate clandestine graves.

The scientific community's growing interest in cadaver decomposition in soil is reflected by the increasing numbers of publications on this topic. Nevertheless, there is still a gap in the knowledge about some key processes, such as human decomposition, soil microbial communities associated with the different decomposition stages, their role and effect on decomposition, and their value as a forensic tool.<sup>1</sup>

This study aims to expand the knowledge base of subsurface decomposition within a forensic context by comparing soil microbial communities associated with the decomposition of mammalian surrogates (*Sus scrofa* domesticus) and plant litter (*Quercus robur*) in a natural setting.

The experimental design consisted of three piglet (*Sus scrofa* domesticus) burials, three plant litter (*Quercus robur*) burials and three soil-only burials (controls). The subsurface decomposition experiment was conducted in a natural setting in secure land in North East England (North Yorkshire, United Kingdom).

Soil pH, soil temperature, atmospheric temperature, and precipitation were monitored, and the associated soils profiled for shifts in bacterial communities by Denaturing Gradient Gel Electrophoresis (DGGE) and high throughput sequencing using 16s ribosomal RNA (rRNA) gene-specific primers. Soil fungal communities were monitored by DGGE using 18s rRNA gene specific primers. Soil samples were collected at regular intervals over a 24-month period. The different treatments were sampled monthly during the first year, then every two months until the end of the study. Sampling was performed at 50cm–60cm deep, without disturbing the buried material. At each time point, four soil samples were collected from around each treatment using a soil corer and combined into a 25ml sterile universal tube, then transported to the laboratory, on ice, and stored at -20°C until required.

The ecological indices calculated after DGGE and data obtained from 16s rRNA high-throughput sequencing allowed a profiling of the soil bacterial and fungal communities at each time point. The results have shown a direct relationship between seasonal temperature changes and shifts in microbial activity, together with exploitable shift patterns with the potential to be used for the estimation of time since death.

Soil microbial communities can be of great value for time-since-death/-burial estimations due to their recognized potential to facilitate higher accuracy by complementing the existing tools.<sup>2</sup> Nevertheless, more research is needed to understand the microbial communities' dynamics in different deposition conditions and their interactions with soil, the cadaver, and its inherent microbiome.

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2. Ralebitso Senior, T. Komang, Tim J.U. Thompson, and Helen E. Carney. Microbial Ecogenomics and Forensic Archaeology: New Methods for Investigating Clandestine Gravesites. *Human Remains and Violence: An Interdisciplinary Journal*, 2, no.1 (2016): 41-57.

#### Cadaver Decomposition, Soil Microbial Communities, Time Since Death



### H100 A Field Study on the Effects of Morphine on the Carrion Decomposition Process and Dipteran Larvae Development

Abigail J. Props, MS\*, Lafayette, IN 47904

**Learning Overview:** After attending this presentation, attendees will learn how morphine affects Dipteran larvae development and carrion decomposition process from a field study in comparison to laboratory research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by looking at morphine effects on carrion decomposition and Dipteran larvae development outside of a controlled laboratory setting.

Laboratory studies have shown that toxicants, in general, can influence the growth and development of maggots.<sup>1-3</sup> These were in controlled environments with fly eggs of specific species being placed on the food source and an infinite food source, in some studies. With taking the research to the field, verification of the laboratory research results can be achieved. Throughout the beginning stages of the blow fly life cycle, larvae are feeding on tissue around the natural orifices and any trauma-created openings. Because not all organs and tissues will have the same concentration of morphine, not all tissue will affect the larvae the same. Lower levels of concentration will result in little to no effect on the growth and development of the Diptera larvae, while tissues with higher levels, such as the liver, are more likely to affect their growth and development.

Current research has shown that morphine has a negative effect on the growth and development of the blow fly larvae—the same effect it has on humans.<sup>3</sup> Morphine can also have a stimulating effect on the growth and development of flesh fly larvae, the opposite effect it has on blow fly larvae and humans.<sup>4</sup> This possible increase/decrease will directly affect the postmortem interval estimation, causing it to be over- or underestimated. Examining these processes will help determine if morphine will affect the postmortem estimation.

This field study looked at the effects of morphine on the growth and development of maggots, colonization rate, and carrion decomposition rate. Five pigs were injected with morphine along with five control pigs being punctured with a needle for five days prior to being euthanized. All ten pigs were allowed to decompose as naturally as possible, in an open field with no vegetation coverage with open exposure to solar radiation. Dipteran larvae were collected in a way that would be comparable to collections at a crime scene. The initial decomposition rate appeared to be faster among the treated pigs as compared to the control pigs but was not significantly different. The larval lengths of individuals belonging to forensically important family, Diptera: Calliphoridae, were not significantly different between the treated and control pigs, in any instars on days three, six, and ten.

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**Entomotoxicology, Morphine, Entomology**



### H101 Building a Microbial Model to Estimate the Postmortem Interval (PMI) Using Data Collected During the Spring Season at Three Anthropological Facilities

*Ariel D. Belk, MS\**, Colorado State University, Fort Collins, CO 80525; *Heather L. Deel*, Fort Collins, CO 80525; *Zech Xu Zhenjiang, MS*, University of California San Diego, La Jolla, CA 92093; *David O. Carter, PhD*, Chaminade University of Honolulu, Honolulu, HI 96816; *Sibyl R. Bucheli, PhD*, Sam Houston State University, Huntsville, TX 77340; *Aaron M. Lynne, PhD*, Huntsville, TX 77341; *Melissa A. Connor, PhD*, Colorado Mesa University, Grand Junction, CO 81501-3122; *Dawnie W. Steadman, PhD*, University of Tennessee, Knoxville, TN 37996; *Giovanna M. Vidoli, PhD*, University of Tennessee, Knoxville, TN 37996; *Rob Knight, PhD*, University of California San Diego, La Jolla, CA 92093-0763; *Jessica L. Metcalf, PhD*, Colorado State University, Fort Collins, CO 80523-1171

**Learning Overview:** After attending this presentation, attendees will understand the changes to microbial community structure that occur after death in human cadavers and how this information can be applied to create predictive models estimating the PMI.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing a novel tool for criminal investigation in cases of unattended death scenes in active and advanced decay.

PMI is crucial for criminal investigation. When known, this metric allows investigators to identify suspects and validate alibis. However, most tools designed for estimating PMI are no longer useful after 48 hours. In these cases of late discovery, the microbes associated with the remains may serve as physical evidence that can be used to estimate the PMI. The communities of microbes and their associated genes, known as the microbiome, have been shown to change in a repeatable manner on decomposing mammalian tissue, therefore providing a microbial clock that can capture the length of the decomposition period. The objectives of this experiment were to: (1) determine if the microbiome changes similarly in different geographic and environmental regions; and (2) use machine learning techniques to build a predictive model of PMI that can be used as a tool in forensic investigation.

Three anthropological research facilities, located in distinct geographic regions in the United States (Colorado Mesa University (CMU), Sam Houston State University (SHSU), University of Tennessee Knoxville (UTK)) were used for this study. Three donated human cadavers were placed at each facility ( $n=9$ ) in the spring of 2016. Skin and soil swabs were collected daily from each body for 21 days of decomposition. DNA was extracted from these samples, and 16S and 18S ribosomal RNA (rRNA) microbial gene amplicons sequenced. Data were then processed to evaluate changes in microbial diversity over time and between locations, and feature information was used to train machine learning models to predict PMI from microbial composition.

Each facility had a distinct microbial signature, primarily driven by the soil composition. As decomposition progressed, the soil came to cluster more closely with the skin samples, likely due to translocation from the cadaver to the soil. The primary phyla found at all sampling points was Proteobacteria, although the relative abundance of Firmicutes increased as decomposition progressed. The changes in microbial diversity and taxonomy that occurred over the 21-day decomposition period differed enough to be included in PMI modeling using machine learning. Overall, in a model built using data from all facilities, the microbiome data could be used to predict the PMI within 3-4 days. Prediction errors from models generated for each facility were similar, with samples predicting PMI within 2-4 days. Each model was also tested across facilities; models trained within a single facility were used to predict PMI at each of the other two facilities. On average, these cross-facility testing errors were within 4.5-6.5 days. The higher error is likely due to temperature ranges in testing sets that were beyond those in the training sets.

Overall, this study confirmed the concept proposed in previous research that the microbiome associated with decomposing mammalian tissue varies in a clock-like pattern. Furthermore, this was then applied to generate predictive models to estimate PMI. These models were accurate, and in future study can be improved by the addition of higher sample numbers and perhaps a longer sampling period. Future work will also include similar results from the summer, fall, and winter seasons to build a fully generalizable model.

#### Postmortem Interval, Microbiome, Machine Learning



### **H102 Using Larvae of Carrion Beetles to Estimate the Time of Death in a Highly Decayed Corpse in Southwestern China: A Case Report**

*Zhou Lyu, PhD\**, Southwest University of Political Science and Law, Chongqing, Chongqing Municipality 0086 401120, CHINA; *Zeying Tang, Master\**, Southwest University of Political Science and Law, Chongqing Municipality 0086 401120, CHINA

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**THIS ABSTRACT WAS NOT PRESENTED.**





### H103 Scanning Electron Microscopy (SEM) in the Identification of Fly Artifacts (FAs): A Preliminary Qualitative Study

Guido Pelletti, MD, Department of Medical and Surgical Sciences, Unit, Bologna, ITALY; Paolo Fais, PhD, Department of Medical and Surgical Sciences, Unit, Bologna, ITALY; Alberto Amadasi, MD\*, Università di Bologna, Bologna 40126, ITALY; Chiara Palazzo, MD, Bologna, ITALY; Maria Carla Mazzotti, MD, Department of Medical and Surgical Sciences, Unit, Bologna, ITALY; Laura Ingrà, PhD, Department of Biomedical and Neuromotor Sciences, Bologna, ITALY; Desiree Martini, PhD, Department of Biomedical and Neuromotor Sciences, Bologna, ITALY; Susi Pelotti, MD, University of Bologna, Bologna 40100, ITALY

**Learning Overview:** After attending this presentation, attendees will understand that SEM is a useful and promising tool to assist in the differential diagnosis between FAs and unaltered bloodstains.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing a key aspect of bloodstain pattern analysis, since many techniques have been applied for the differential diagnosis between bloodstains and FAs, but most of them rely on the experience and opinion of the analyst rather than on standardized and reproducible methods. Morphological analysis through SEM may provide reliable objective parameters to daily forensic casework.

Bloodstain Pattern Analysis (BPA) has a key role in crime scene investigation. After deposition, bloodstains can be altered by diverse forces, some of which are related with the crime, while others may act as confounding factors, such as the stains produced by insect activity, which are commonly referred to as FAs. Flies can produce FAs in different manners: by contact, by regurgitation, and by defecation. Differential diagnosis between FAs and bloodstains is sometimes cumbersome, especially in cases of small stains (0.1cm–0.3cm). Several techniques and methods of analysis have been used to differentiate between unaltered bloodstains and FA, such as visual macro-microscopic and contextual analysis, heme-based presumptive tests and immunological confirmatory tests. However, these techniques mostly rely on the experience and opinion of the analyst rather than on standardized and reproducible methodology.

The present survey aimed at testing the potential utility of SEM for distinguishing bloodstains from FA produced by *Sarcophaga carnaria* under experimental conditions.

One hundred adults of *Sarcophaga carnaria* were placed in a scaled-down room analog with free access to 50ml of fresh human blood and were free to deposit fly artifacts on five different surfaces (two porous and three non-porous types of paper) for 48 hours. Experimentally produced bloodstains were used as controls. FAs and controls were compared through visual analysis (color, shape, tail, edges) and SEM analysis (surface, deposits, presence of red blood cells) analysis.

Visual analysis allowed the identification of two types of FAs. Type 1FAs (FAs1) showed brownish color with no or short tails and resembled the controls, while Type 2FAs (FAs2) showed yellow-brownish color with longer and curved tails, being easily distinguishable from controls and FAs1. SEM analysis allowed the distinguishing between controls and FAs through the identification of red blood cells on the surface of the controls, which were absent in both types of FAs. Other distinctive morphological features were observed: (1) FAs1 showed luminescent deposits on the surface, which were absent on controls and FAs2; and (2) FAs2 showed irregular/glomerular surfaces, while controls and FAs1 showed flat or cratered surfaces.

Based on these features, the detection through SEM of red blood cells on the surface of the controls was the key element for the differentiation between FAs and bloodstains, since red blood cells were present in all controls and absent in all FAs. Other features can be considered to confirm the differential diagnosis, such as the presence of luminescent deposits and the morphological features of the surface of the spot.

Therefore, SEM analysis demonstrated to be a useful tool for the distinction between FAs and unaltered bloodstains, since it allowed identification of some objective morphological features that do not rely on subjective evaluation. In the future, this technique could be used for the differentiation of FAs and bloodstains deposited by different species on other types of surfaces to assess the potentialities of this technique in real forensic scenarios.

#### Fly Artifacts, Bloodstain, Scanning Electron Microscopy (SEM)



### H104 Forensic Imaging in Two Cases of Survived Car Surfing in Zurich, Switzerland

*Michela Lanfranchi\**, Zurich Institute of Forensic Medicine, Zurich 8057, SWITZERLAND; *Michael Thali, MD\**, Universitat Zurich, Zurich CH-8057, SWITZERLAND; *Wolf Schweitzer\**, Universitat Zurich, Zurich CH-8057, SWITZERLAND

**Learning Overview:** The goal of this presentation is to demonstrate the use of clinical Computed Tomography (CT) in forensic reconstruction of car surfing and in the differentiation of the presented injuries in the overall context containing different possibilities for injury causation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enabling attendees to appreciate the power of dedicated forensically reconstructed CT images based on clinical CT images in conjunction of conventional photographic documentation in the specific context of car surfing.

**Proposition:** That clinical CT is highly relevant in the forensic reconstruction of survived car surfing victims.

**Introduction:** Car surfing describes the ride that a person experiences or performs when holding onto the outside of a passenger car, which is not legally approved on public roads. The discrimination of injuries as consequence of car surfing appear to be of particular legal, and therefore forensic relevance, not only because of injury severity but also the risk that has to be considered and assessed. With the insight and experience of the Virtopsy project, which now overlooks a time span of 25 years with forensic Postmortem Computed Tomography (PMCT), applying that knowledge based on forensic postmortem imaging to clinical cases is an increasing focus in forensic medicine.

**Cases:** This case series contains two cases of car surfing that were investigated by this study. In both cases, forensic physicians from the Zurich Institute of Forensic Medicine performed a physical examination of the victim with a forensic scientist. The examination was supplemented with a retrospectively added forensic assessment of high-resolution CT images that had been acquired in the respective emergency departments where the victims had been admitted.

**Results:** The two unrelated cases both featured male car surfers in their early thirties, found riding on the hood of a car. Both car rides appeared to have been escalations of a preceding verbal and physical argument. In the first case, forensic examination was performed approximately six hours after the event. The driver of the car was an enraged taxi driver, and the car surfing ride was alleged to have reached up to 125kmph (77mph). In the second case, forensic examination took place 13 hours after the event. There, the driver was an allegedly petrified escort girl, in which the driving speed was not known but the glass of the windshield had sustained fractures. In both cases, the individuals were severely injured after they had fallen off the hood. They had to be brought to the local hospital emergency departments. In the first case, with the head, torso, and extremities injured, the most severe trauma was to the feet, with a very significantly abraded left great toe. Two lacerations on the head had to be surgically treated with stitches. The second case featured several fractures to the skull, and the clinical course was primarily defined by the severe blunt head trauma. There also were abrasions to the upper extremities typical for finger nails. Forensic questions into both cases contained differentiation between injuries sustained from a physical fight, from those sustained while car surfing, from those sustained as consequence of falling from the car.

**Conclusions:** In both cases, CT imaging was relevant from a forensic point of view for event reconstruction. The victim in the first case sustained a relatively serious toe injury and two lacerations of the head that required surgical repair. In particular, the second case benefited both from extensive visualization of the skull fracture patterns that were interpreted and with comparison against the forensic photographs of the car. In this presentation, both cases will be presented with extensive images of both CT and ancillary photography.

#### Virtopsy, Car Surfing, Forensic Imaging



### H105 Postmortem Computed Tomography (PMCT) Versus the Skeletal Survey in Children: A Pathology-Proven Study

*Esther L. Van De Mortel, MSc, Master's Programme in Forensic Science, Faculty of Amsterdam, Noord-Holland, NETHERLANDS; Judith Fronczek, MD, PhD, Department of Forensic Medicine, Netherlands Forensic Institute, The Hague 2497 GB, NETHERLANDS; Simon G.F. Robben, MD, PhD, Department of Radiology, Maastricht University Med, Maastricht 6229 HX, NETHERLANDS; Rick R. Van Rijn, PhD\*, Amsterdam UMC, Amsterdam 3544MT, NETHERLANDS*

**Learning Overview:** After attending this presentation, attendees will understand the value of skeletal surveys and PMCT in deceased children with respect to the detection of fractures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing a potential new approach to postmortem pediatric imaging in cases of suspected physical abuse.

Annually, between 118,000 and 180,000 children in the Netherlands are exposed to some form of abuse. Of these children, 18% are physically abused. It has been shown that child abuse not only has short-term but also long-term effects on the (mental) health of a child. Therefore, it is important to detect child abuse as early as possible to protect the health of the child and to prevent further injuries, of which death is the most extreme outcome. To do this, non-accidental injuries should be recognized correctly by the physician and an imaging technique should be available to accurately detect abuse-related injuries. In children under the age of 3 years, a skeletal survey is a mandatory procedure in the work-up of suspected physical abuse. This skeletal survey consists of a predefined set of Conventional Radiographs (CR) as defined by the Royal College of Radiologists and the Society and College of Radiographers.

In recent years, several studies in adults have shown that Computed Tomography (CT) has a higher sensitivity for rib fractures and skull fractures, for example. However, it is not clear if this is true for children as well, or if CT has the ability to detect small metaphyseal corner fractures.

The goal of this research was to determine whether it was possible to (partly) replace the skeletal survey by PMCT. For this determination, deceased children younger than five years referred to the Netherlands Forensic Institute for a forensic autopsy in whom both a skeletal survey and a PMCT scan were obtained were included in this study. Included cases were retrospectively reviewed by an experienced pediatric radiologist. The fracture patterns on CR and CT were scored and evaluated. Out of 55 eligible cases, fractures were observed in 24 cases (16 boys (mean age: 3.25 months, age range: 1–8 months) and 8 girls (mean age: 3.25 months, age range: 0–13 months). Radiology findings were compared to full autopsy reports.

CR and CT were concordant in 36 cases and non-concordant in 19 cases. It was shown that CT detects significantly more rib fractures compared to CR or autopsy ( $p=0.01$  and  $p=0.004$ , respectively). Also, CT detected more skull fractures compared to CR. In contrast, CR had a higher sensitivity compared to PMCT (e.g., metaphyseal corner fractures), where PMCT missed two out of three fractures. Autopsy was superior in detecting basilar skull base fractures ( $N=5$ ), which all were missed by CT and CR.

These results suggest that radiographs of the thorax and skull can be excluded from the skeletal survey in a postmortem setting. However, it is shown that PMCT cannot completely replace the skeletal survey, as clinically relevant metaphyseal corner fractures were missed on CT. Based on these findings, it is suggested that a whole body PMCT with additional radiographs of the joints, hands, and feet may be a viable option in a forensic postmortem setting.

#### Child Abuse, Fractures, Radiology



### H106 Upper Versus Lower Limb Pediatric Fracture Healing: A Radiographic Study of Time Since Injury (TSI)

Diana L. Messer, MS\*, Defense POW/MIA Accounting Agency, Joint Base Pearl Harbor-Hickham, HI; Brent H. Adler, MD, Nationwide Children's Hospital, Columbus, OH 43205; Farah W. Brink, MD, Nationwide Children's Hospital, Columbus, OH 43205; Huiyun Xiang, MD, PhD, Nationwide Children's Hospital, Columbus, OH 43205; Amanda M. Agnew, PhD, The Ohio State University, Columbus, OH 43210

**Learning Overview:** After attending this presentation, attendees will understand the influence of fracture location on pediatric fracture healing and its potential impact, in both forensic and clinical contexts, for radiographic methods of TSI estimation, particularly in cases of child physical abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting that not only should future TSI methods be specific to fracture location, but that the utility of methods derived from pooled bone samples may be inaccurate and inappropriate for use in a forensic or clinical setting. Improved TSI methods may lead to more accurate identification of child physical abuse that can inform decision-making regarding child protection.

The relationship of fracture location to healing is vastly understudied and its potential impact on TSI estimates remains largely unknown. As a result, timelines of pediatric fracture healing are often based on pooled samples of skeletal elements, which may not provide accurate results if healing rates differ significantly by location. Malone et al. suggest differences in healing between fractures of the radius and tibia, but whether this applies to all bones of the upper and lower limb remains unknown, and few, if any, existing methods take fracture location into account.<sup>1</sup>

This study assesses radiographic features of fracture healing in a modern pediatric sample of accidental fractures to evaluate whether fracture location (upper vs. lower limb) significantly influences rate of fracture healing.

Four hundred ninety-eight skeletal fractures (>1,355 radiographs) at Nationwide Children's Hospital in Columbus, OH, were evaluated for features of fracture healing, specifically fracture margin definition, Subperiosteal New Bone Formation (SPNBF), and callus formation. Features of SPNBF and callus were recorded for presence, thickness, matrix, and character based on modified parameters set by Walters et al.<sup>2</sup>

Independence and goodness of fit frequency tests were used to evaluate the relationship between fracture age and levels of SPNBF, callus formation, and fracture margin definition. Analysis of covariance tests were conducted to control for patient age, which has been demonstrated to affect fracture healing.<sup>3</sup>

Results of this study demonstrate that limb location significantly affects SPNBF thickness ( $p < 0.001$ ), with SPNBF occurring earlier in upper limb fractures. In addition, SPNBF thickness that was greater than 75% of the original bone shaft width only occurred in upper limb fractures. Limb location also significantly affects SPNBF character ( $p < 0.05$ ), with single-layered and multi-layered SPNBF occurring earlier in upper limb fractures. Further, limb location significantly affects callus thickness ( $p = 0.002$ ), with callus formation appearing thicker earlier, and lasting for a shorter duration, in upper limb fractures.

*This research was supported by the Center for Injury Research and Policy at Nationwide Children's Hospital through a grant from the Centers for Disease Control and Prevention. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention and the Center for Injury Research and Policy.*

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#### Fracture Healing, Child Abuse, Forensics



### H107 Virtopsy in Clinical Forensic Medicine: A Trauma Case Series With Clinical Emergency Computed Tomography (CT) Reconstruction Details

Eddie Surer, MD\*, Zurich Institute of Forensic Medicine, University, Zurich 8057, SWITZERLAND; Michael Thali, MD\*, Universitat Zurich, Zurich CH-8057, SWITZERLAND; Rosa M. Martinez, MD\*, Universitat Zurich, Zurich CH-8057, SWITZERLAND; Valeria Hofer\*, University of Zurich, Zuerich CH-8057, SWITZERLAND; Michela Lanfranchi\*, Zurich Institute of Forensic Medicine, Zurich 8057, SWITZERLAND; Wolf Schweitzer\*, Universitat Zurich, Zurich CH-8057, SWITZERLAND

**Learning Overview:** The goal of this presentation is to explain in detail how image quality and resolution of clinical emergency CT images serves to solve forensically relevant questions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how good emergency CT data is considering forensic analysis of even relatively small survived injuries.

**Introduction:** Emergency CT images have a therapeutic focus, and axial images have been the mainstay of their interpretation. That data has been regarded as inadequate for forensic questions as reconstructive forensic approaches also heavily employ Multiplanar Reconstruction (MPR), and 3D techniques demand sufficient resolution also of the third dimension (z-axis, slice distance). This case series provides examples of the forensic use of that data.<sup>1</sup>

**Cases:** Case 1 features blunt head trauma of a bicycle rider found with a nose bleed and visible facial injuries but no bicycle. The forensic question was to discriminate between blows against head and a fall. Case 2 features a sternal stab wound with the forensic question of why, as there were more than 20 mostly very superficial stab wounds allegedly delivered by a single person, there was no other fracture. Case 3 features a firearm injury to the head with the forensic question why the bullet exited with deformation, seeing as there was a straight line from entrance (right neck) to exit (left eye) that did not contain thick bone. Case 4 features multiple stab wounds to head and torso with questions as to how deep and how close to vital structures they were. CT scan details were accessed through Digital Imaging and Communications in Medicine (DICOM) -header tags.

**Results:** Resolution of clinical emergency CT scans was 0.35mm to 0.46mm inplane (x/y) and 0.5mm to 0.7mm (z) for head scans and 0.88mm to 0.97mm (x/y) with 1mm to 1.5mm (z) for thorax and abdomen scans. Anisotropy (percentage: z relative to x/y) was 141%±36%. The time required to acquire data to reconstruct a head CT at maximal z-resolution of 0.5mm was 26 seconds, not that different from a head scan with less z-resolution (such as 0.7mm), where the longest duration in this case series was 33 seconds. Thorax CT scans took 6 to 10 seconds with substantially larger image thicknesses.

In Case 1, burst fracture lines, some of them quite thin, showed a combined burst and impression fracture system to the face typical for severe blunt trauma, such as riding a bicycle into a street sign at relatively high speed, matching the victim's statement. In Case 2, features of both a sternal foramen and sharp force injury to the bone could be discriminated, explaining why a relatively superficial stab wound injury pattern was not a contradiction to a perforated sternum. In Case 3, the bullet track also containing a ricochet area at the orbital rim of the left eye containing a subtle hairline fracture could be visualized in detail. In Case 4, all deep stab injuries could be reconstructed to answer regarding their depth and proximity to vital structures.

**Discussion:** Clinical emergency CT uses similar machines as those used for Postmortem CT (PMCT), so they have no intrinsically lower data quality. In all these cases, visualizations were achieved that answered the forensic questions not only with plausibility but with confidence. While resolution of CT scanners generally is sufficient for many forensically relevant injuries, even limited resolution or quality of radiological images has been shown to be relevant in the context of forensic medicine in the past.<sup>2,3</sup>

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**Virtopsy, Clinical Forensic Imaging, CT Imaging**



### H108 My Darkest Hour: Bringing a Consumer LED Torch Light Into Forensic Macrophotography Through 3D-Design of a Lamp Mount for a Consumer Point-and-Shoot Camera

Wolf Schweitzer\*, *Universitat Zurich, Zurich CH-8057, SWITZERLAND*; Valeria Hofer\*, *University of Zurich, Zuerich CH-8057, SWITZERLAND*; Rosa M. Martinez, MD\*, *Universitat Zurich, Zurich CH-8057, SWITZERLAND*; Michael Thali, MD\*, *Universitat Zurich, Zurich CH-8057, SWITZERLAND*

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**Learning Overview:** After attending this presentation, attendees will understand how a mounted Light-Emitting Diode (LED) can result in great forensic macroscopy photos with a point-and-shoot consumer camera.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how extremely affordable materials (3D-printed lamp mount, LED torchlight) can cause massive quality improvements in forensic macrophotography.

Macrophotography contains an application domain with an extremely limited parameter space circled by sensor size, aperture, exposure time, and International Organization for Standardization (ISO) number. This study pragmatically asks how to proceed to achieve cost-effective equipment for around ten assistant doctors of a medicolegal institute to perform optimal hand-held “action” shots on death scenes or event scenes or during clinical forensic medicine examinations. The benchmark lesion is the single conjunctival pinpoint hemorrhage as encountered after strangulation.

**Analysis, Methods, and Materials:** High aperture numbers (i.e., small opening) promise great depth-of-field and not only reduce but also diffract light, which lowers image quality, particularly on small sensors. Reduced light is remedied with higher ISO numbers that increase visual noise. Increasing exposure time risks motion blur. Despite optimizing these parameters, image quality remains severely restricted. This study fitted an LED lamp (CREE-XML-T6, ~\$2 USD) on a 3D-designed hot-shoe fitted mount with an interlocking joint (3D-printed PLA ~\$1.50 USD, screw assembly ~\$2.20 USD) on the consumer camera (Canon® G16, modified firmware to read out scene illumination in  $L=cd/m^2$ ), yielding a total cost of the LED lamp including Mount (LEDMNT) of ~\$4.20 USD (not counting batteries). This study compared images in realistic settings photographed: (1) with no extra light; (2) using LEDMNT; and (3) the integrated as well as a hot-shoe mounted Canon® Speedlite 270EXII flashlight.

**Results:** A first series of 20 photos of skin injuries photographed postmortem at normal autopsy room ceiling light showed that luminance measured on the sensor at a medium range ( $28cd/m^2$ – $60cd/m^2$ ) dropped to  $2cd/m^2$ – $10cd/m^2$  for close range macro photos with no extra light source (1). Using the LEDMNT (2) values of  $90cd/m^2$ – $500cd/m^2$  were achieved while flashlight (3) caused significant shadows in close-range macro photos. The image quality improved drastically after installing the LEDMNT.

**Discussion:** It is not a new insight that more light can improve photographs. However, this contribution is not theoretic but practical and represents an extremely affordable and practical approach to a very relevant problem.

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#### Virtopsy, Forensic Macro-Photography, 3D Printing



### H109 Role of Postmortem Computed Tomography (PMCT) as a Triage Tool Between External Inspection and Full Autopsy

Vasiliki Chatzaraki, MD\*, Institute of Forensic Medicine Zurich, Zurich CH-8057, SWITZERLAND; Jakob Heimer, MD, Universitat Zurich, Zurich CH-8057, SWITZERLAND; Michael Thali, MD, Universitat Zurich, Zurich CH-8057, SWITZERLAND; Wolf Schweitzer\*, Universitat Zurich, Zurich CH-8057, SWITZERLAND

**Learning Overview:** The goal of this presentation is to show the current advantages and weaknesses of PMCT usage in the forensic pathologist's routine. As imaging has been expanded around the world as a supplement to conventional autopsy, it is important to consider the strengths as well as the limitations and pitfalls.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the strengths of PMCT regarding determining the cause and manner of death and excluding or indicating signs of violence. However, there are also limitations in PMCT usage, which may be solved by increasing the quality of image acquisition and doing more research in the field. Combining imaging with toxicological analysis and biopsies is also recommended.

**Objectives:** In forensic medicine, PMCT and PMCT Angiography (PMCTA) assist in determining cause and manner of death, providing significant information regarding specific forensic questions.<sup>1-3</sup> After the external examination of a body, PMCT appears to be a valuable tool for imaging-based decision making, whether or not conducting an autopsy is necessary. The role of PMCT has become increasingly interesting as a method to improve decision making rather than a one-stop solution for all forensic morphological postmortem issues. Its costs can be significantly lower than those of a conventional autopsy, and the information gained can be significant. Its relevance is dependent on the specific forensic question; however, that forensic question is always case dependent. With that, not all that is visually apparent on PMCT also forensically solves every case. Within that domain of application, a series of ten cases is presented to qualitatively highlight the advantages and remaining problems of the current PMCT usage.

**Materials:** Due to their relevance for the question of decision making, a deliberate and manually selected case series of ten PMCT examinations was selected from the Institute of Forensic Medicine Zurich's database (time range 2014 to 2017). All cases had been admitted to the Institute following a forensic death scene investigation for further examination and assistance in judicial decision making. In five of the cases, a forensic autopsy was conducted after imaging. One case underwent PMCTA in addition to unenhanced PMCT. In one case, a CT-guided lung biopsy was performed. In eight cases, toxicological analyses of body fluids (qualitative toxicological screening within PMCT triage or quantitative after collecting blood and urine during autopsy) were conducted. Time interval between death and PMCT was 14.9h±12.8h (range: 3h to 48h). All case protocols were evaluated for the final manner and cause of death.

**Results-Conclusion:** Five cases were released after imaging without conducting an autopsy as PMCT-PMCTA alone or combined with toxicological screening or lung biopsy confirmed a cause and manner of death and/or excluded third-party involvement/malpractice or ensured identity, answering the relevant forensic questions required. In one case, PMCT revealed a large number of tablets in the stomach, supporting the already-assumed cause and manner of death; however, toxicological analysis did not match the drug suspected according to scene inspection. In another case, PMCT failed to reveal laryngeal fractures indicating violence and possible homicide. In the other two cases, PMCT indicated infectious disease without being able to confirm a microbiological diagnosis; however, it warned the pathologists to protect their health during autopsy.

PMCT can satisfactorily answer the leading forensic questions of some cases alone, like the validation of the victim's identity, the exclusion of third party violence, and the presence of foreign bodies and determine the cause and manner of death. Thus, a conventional medicolegal autopsy can be avoided as all relevant forensic questions are answered, which is also time and cost consuming. However, reliable evaluation of relevant head and cervical trauma, strangulation, infection and sepsis, metabolic disorders, intoxication, and neuroreflex deaths keeps evading a purely PMCT-based approach.<sup>4,5</sup>

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#### Virtopsy, Triage Tool, Postmortem Computed Tomography



### H110 Postmortem Fetal Angiography: A Tool for Better Understanding Congenital Vascular Malformations

*Jessica Vanhaebost\**, Brussels, BELGIUM; *Xavier De Spiegeleire, Bac*, Cliniques Universitaires saint-Luc, Brussels 1200, BELGIUM; *Gregory Schmit, MD*, Service de Médecine Légale, Brussels 1200, BELGIUM; *Catherine Behets, PhD*, Université Catholique de Louvain - Pole of Morphol, Brussels 1200, BELGIUM; *Emmanuel Coche, PhD*, Cliniques Universitaires Saint-luc, Brussels 1200, BELGIUM

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**Learning Overview:** The goal of this presentation is to adapt postmortem angiography to a small-caliber vascular system, as found in the fetus, and to evaluate the feasibility of the fetal postmortem angiography in a routine clinical examination. After attending this presentation, attendees will understand the feasibility and impact of the intravascular injection of contrast medium to detect vascular malformations. Preliminary results will be shown and discussed with attendees.

**Impact on the Forensic Science Community:** Few articles have been published on this topic or how to handle this type of forensic question. The injection technique, involving an oily contrast agent dedicated to postmortem examination using a multislice Computed Tomography (CT) scan has seemingly never before been performed in a fetus. Therefore, this presentation will impact the forensic science community by providing a methodological approach to this medicolegal issue. This method will also impact the forensic community in cases of untimely intra-uterine death, which raises questions about the obstetric management of pregnancy and may lead to complaints from parents.

**Method and Material:** In order to demonstrate feasibility, ten fetuses (fresh and frozen) were investigated by whole-body postmortem CT scanner with the following parameters: slice thickness: 0.4 mm; field of view: 25cm, 80kV, and 168mAs, using a multislice CT scan (Definition 40) in single-energy mode, without and after intravascular injection (very low flow) of contrast medium dedicated to postmortem examination (Angiofil®) by umbilical or transcardiac injection under ultrasound control. The images were reconstructed on a workstation using a dedicated software.

**Results:** The results show the feasibility of injection and opacification of the entire fetal vascular system without any tissue extravasation in a very important postmortem delay (up to two years after death) by umbilical vessels. The amount of contrast agent necessary to opacify the entire vascular system is approximately 15mL. The time required to obtain an access route for the injection was essentially dependent on the state of preservation of the fetus. Arteries and veins of large caliber and infra-millimeter vessels could be visualized during the CT examinations.

**Conclusion:** This study demonstrates the feasibility of postmortem angiography of the fetus and its ability to opacify very small visceral vascular structures on multi-slice CT. Further investigations, especially autopsy, are needed to compare the findings side-by-side and understand the clinical and medicolegal implications of such imaging.

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### Fetal, Angiography, Vascular Malformation





### H111 The Value of Craniocervical Magnetic Resonance Imaging (MRI) Following Non-Fatal Strangulation

Jakob Heimer, MD\*, *Universitat Zurich, Zuerich CH-8057, SWITZERLAND*; Carlo Tappero, *Institute of Forensic Medicine, Zurich, SWITZERLAND*; Dominic Gascho, *Universitat Zurich, Zurich CH-8057, SWITZERLAND*; Patricia Flach, PD, *Kantonsspital St. Gallen, St. Gallen, SWITZERLAND*; Thomas Ruder, PD, *Northland District Health Board, Whangarei, NEW ZEALAND*; Michael Thali, MD, *Universitat Zurich, Zurich CH-8057, SWITZERLAND*; Sabine Franckenberg, MD, *Diagnostic and Interventional Radiology, Zurich 8091, SWITZERLAND*

**Learning Overview:** After attending this presentation, attendees will be updated on the current state of magnetic resonance imaging following non-fatal strangulation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing a well-founded idea of when MRI following non-fatal strangulation is worth logistic and financial efforts.

**Introduction:** Manual strangulation is a form of assault frequently encountered in the context of domestic violence targeted against women. Manual strangulation results in a wide range of health impairments, including death. At the same time, external findings are often absent or scarce. To reveal possible internal injuries not observable in external documentation, MRI was introduced to the forensic examination of strangulation survivors a decade ago. Both Yen et al. and Christe et al. described various findings related to the assault, and suggested MRI to be a worthwhile asset in the medicolegal investigation.<sup>1,2</sup> The University of Zurich has offered MRI to every victim of manual strangulation since 2011. Based on a database of 114 completed MRI examinations at the time, this study present results on the value of craniocervical MRI following manual strangulation.

**Methodology:** All cases were routinely examined including a full documentation of case history and photographic documentation of external findings. Cases were then invited to undergo craniocervical MRI and were issued a gratification card for free transport to the clinical examination center. MRI was conducted as fast as possible with dedicated sequences. Radiologic images were evaluated by a clinical radiologist and a forensic radiologist. Case data was grouped into external findings (hematoma, abrasions, swelling), subjective findings (localized pain, difficulty swallowing and/or breathing, loss of urine and/or feces, loss of consciousness) and MRI findings (sub-cutaneous, intramuscular, and peri-laryngeal fluid accumulations, lymph node hemorrhage).

**Results:** The case group consisted of 94 women and 24 men with a mean age of 32.5 years. The most prevalent methods of attack were both-handed (41.1%) and one-handed (41.2%), followed by chokeholds (12.3%), and manual ligature strangulation (4.4%). More men than women were victims of chokeholds. Visible external findings were present in 106 (93%) cases. Subjective findings were reported by 95 cases (83.3%). MRI yielded positive findings in 49 (43%) cases, with a total number of 75 radiologic findings. In 7 cases, MRI was positive while no external findings were delimitable. MRI findings of (peri-)laryngeal structures were significantly associated with reported difficulty swallowing and chokeholds. In only one case did cranial MRI yield a positive result, but an association with the assault could not be safely established.

**Discussion:** The additional value of craniocervical MRI in victims of non-fatal strangulation is limited to victims of severe attacks and reported subjective symptoms, when no or few external findings are delimitable. For a low sensitivity-reproduction of superficial fluid accumulations, logistical and financial efforts of MRI appear not to be justified. The stark contrast to the number of findings of previous studies calls for a common definition and standardization of radiological findings. The acquisition of cranial MRI is not feasible, unless it is routinely indicated by the presence of neurological symptoms.

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#### Domestic Violence, Strangulation, Magnetic Resonance Imaging



### H112 Evaluation of Arterial Injuries Using Contrast and Conventional X-Rays: A Practical Method for the Assessment of Arterial Injuries in Forensic Settings

*Nikki Mourtzinou, DO\**, Office of Chief Medical Examiner, Baltimore, MD 21204; *Zabiullah Ali, MD*, Office of Chief Medical Examiner, Baltimore, MD 21223; *David R. Fowler, MD*, Office of Chief Medical Examiner, Baltimore, MD 21223

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**Learning Overview:** After attending this presentation, attendees will understand the utility of using contrast injection and conventional radiography to identify and diagnose vascular injury in a routine autopsy setting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a practical and inexpensive method to identify vascular injuries during autopsy.

Postmortem vascular visualization, using various contrast agents, has been in use since the 17<sup>th</sup> and 18<sup>th</sup> centuries to study vascular anatomy. With advances in radiology, Multidetector Computed Tomography (MDCT) has been increasingly used for vascular and soft tissue imaging in forensic settings. The use of MDCT angiography is more common in European countries, but due to excessive costs, only a handful of medical examiner offices in the United States are equipped with MDCT. As a result, most medical examiner offices continue to use conventional radiography to aid in the assessment of vascular pathology in forensic settings. At the Office of the Chief Medical Examiner (OCME) for the State of Maryland, MDCT is available and has proven a useful tool in the assessment of traumatic injury at autopsy, although time constraints and/or staff training impedes the practical use of MDCT on a consistent basis. This study performed targeted arterial angiography as an adjunct to routine autopsy using conventional radiography. In forensic autopsies, identification and dissection of vascular regions can prove critical in determining a cause of death, but in many cases, access to these regions can prove time consuming, invasive, and at times, impossible. A goal of this study was to evaluate these hard-to-access vascular regions that remain cumbersome for forensic pathologists, especially in a busy metropolitan office. Another objective of this study was to concentrate on the practical and logistic aspects of visualizing the vascular anatomy and associated trauma, which could be used daily in a busy medical examiner's office.

Although numerous different contrast media are available for use in clinical and postmortem angiography, this study achieved excellent results using a water-soluble, barium-based contrast agent that is easily obtainable with minimal cost implications. To achieve better imaging quality, the vessels were flushed prior to contrast injection using tap water to remove any air bubbles or postmortem clots. There was no difference between using saline or regular tap water for flushing of the vessels.

Study cases were selected based on the likely presence of visualizing vascular trauma and included gunshot wounds, stab wounds, and motor vehicle/pedestrian injuries. In some cases, visualization of vascular injury was well-correlated with subtle gross autopsy findings that may otherwise have been overlooked. This study demonstrates that using contrast injection combined with conventional radiography provides medical examiners with an additional and inexpensive tool for assessing vascular injuries and associated trauma during autopsy.

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**Contrast Agent, Conventional X-Rays, Vascular Injuries**



### H113 The Needle in the Haystack: Histology of Postmortem Computed Tomography (CT) -Guided Biopsies Versus Autopsy-Derived Tissue

*Bartholomeus G.H. Latten, MSc\*, Nederlands Forensisch Instituut, Den Haag, Zuid Holland 2497 GB, NETHERLANDS; Frans Bakers, Maastricht University Medical Centre, Maastricht, Limburg 6229HX, NETHERLANDS; Paul Hofman, PhD, Maastricht University Medical Center, Maastricht 6202 AZ, NETHERLANDS; Axel zur Hausen, MD, PhD, Maastricht University Medical Centre, Maastricht, Limburg 6229 HX, NETHERLANDS; Bela Kubat, PhD, Nederlands Forensisch Instituut, Den Haag, Zuid Holland 2497GB, NETHERLANDS*

**Learning Overview:** The goal of this presentation is to make a critical statement concerning the inferiority of histological examination of postmortem CT-guided biopsies in comparison to tissue derived during the classic autopsy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the limitations of postmortem imaging/minimal invasive autopsy and raising reasonable doubts concerning new non-validated techniques.

**Objective:** During the last decade, autopsies have been declining worldwide, whereas non-invasive methods have been proposed as an alternative for the classic autopsy. Some of these methods add needle biopsies to imaging techniques to provide an alternative to histological examination of autopsy-derived tissue. The goal of this study was to investigate the representativeness of the histology of CT-guided postmortem needle biopsies in comparison to autopsy derived tissue from the same organ.

**Method:** Consent of next of kin was obtained from relatives of the deceased within the department of Internal Medicine to perform an autopsy, postmortem CT, and CT-guided needle biopsies. The lungs and the liver were routinely sampled with CT-guided postmortem biopsies and during the autopsy. In addition, optionally extra CT-guided biopsies of lesions reported on the CT were sampled. The biopsy and the autopsy reports were independently reported and retrospectively coded according to the Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands (PALGA). Three pathologists with an interest in autopsy pathology and three physicians of the department of internal medicine separately and independently interpreted all histological results in relation to the cause of death as formulated in the autopsy report. Fleiss's Kappa was calculated, and a consensus grade was defined.

**Results:** Fleiss's Kappa showed substantial agreement in both lungs and moderate agreement in the liver. Of the 60 included cases, 43% of the CT-guided postmortem biopsies in the left lung and 30% in the right lung showed false negative findings, primarily concerning a bronchopneumonia. In contrast, 91% of the liver biopsies showed concordant results; however, only 22% of these concerned a major diagnosis related to the cause of death. The positive predictive value of the biopsies of the left lung, right lung, and the liver were, respectively, 86.8%, 88.9%, and 100%. The negative predictive values of these biopsies were 34.3%, 50% and 92.7%, respectively.

**Conclusion:** CT-guided postmortem biopsies of the lungs have a mediocre predictive value. Due to a low prevalence of relevant findings, the overall usefulness of biopsies of the liver is limited. Conventional autopsy should still be preferred to biopsy-based postmortem examination. Postmortem biopsies are only an alternative if consent for an autopsy cannot be obtained.

#### Biopsies, Autopsy, Histology



### H114 Deep Learning on Volumetric Radiologic Imaging Datasets for Forensic Pathology Investigations

*Summer J. Decker, PhD\*, University of South Florida, Tampa, FL 33606; Lars C. Ebert, PhD\*, University of Zurich, Zurich CH-8057, SWITZERLAND; Jonathan M. Ford, PhD, University of South Florida, Tampa, FL 33606-3571; Akos Dobay, PhD, University of Zurich, Zurich CH-8057, SWITZERLAND; Natalie L. Adolphi, PhD, Center for Forensic Imaging, Albuquerque, NM 87131; Kurt B. Nolte, MD, Office of Medical Investigator, Albuquerque, NM 87131-0001*

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**Learning Overview:** After attending this presentation, attendees will understand: (1) the concepts of Artificial Intelligence (AI) and Deep Learning (DL), (2) the hardware and software needs, (3) the potential impact of automated analysis on Postmortem Computed Tomography (PMCT) imaging, and (4) the greater role AI algorithms such as DL will play for forensic pathology in the future.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the potential utility of DL as an automated triage tool for PMCT.

Traditionally, Forensic Pathologists (FP) perform an external examination, then document internal pathologies via the autopsy. Imaging modalities, such as PMCT, can establish the cause of death in certain types of cases and better elucidate specific traumas. Postmortem medical imaging also has the added value of helping with teaching, expert witness demonstration, quality control, and telemedical consultation. The flexibility of medical imaging can assist an already-overtaxed Medical Examiner (ME) system as either a tool for triaging cases or supplementing the autopsy process. The growing opioid crisis in the United States has dramatically increased the number of autopsies needed and threatens National Association of Medical Examiners (NAME) accreditation compliance for many offices based on excessive workload. Pathologists attempting to accommodate these expanded caseloads without adequate support can become fatigued and prone to error.

While PMCT can significantly reduce the burden on offices, FPs still may not have adequate time to search for more routine but tedious findings, such as the thorough detection of bone fractures or volume estimation of organs. To further reduce time and costs for interpreting images generated, assistance systems for image reading are required. Current advances in clinical radiology are being made toward adopting DL for patient care. Studies are showing the impact of DL in areas such as the identification of lung nodules and automated brain tumor segmentation. Similar studies must be undertaken to enable use of this technology with respect to forensics. As postmortem scans have unique findings that do not occur in images from living patients, existing DL techniques will require some modifications. Postmortem imaging datasets tend to be larger, have a greater number of images and different scanning protocols, detect different pathologies and artifacts in addition to other complications, such as decomposition and extensive polytrauma.

Clinical radiology has already made significant headway in leveraging DL as a triage and first-pass diagnostic tool. However, medicolegal postmortem scanning has unique requirements and, consequently, specific algorithms need to be created. This presentation will demonstrate the use and potential value of DL in analyzing, screening, and triaging PMCT scans to evaluate its potential as a forensic tool. This presentation will provide examples of how a DL workflow is set up as well as representative examples of how DL can tackle specific forensic pathology problems.

As forensic pathologists in the United States continue to adopt advanced radiologic imaging, tools such as DL need to be in place to improve the overall throughput of the medicolegal death investigative process. AI and DL have the potential to address the diagnostic questions facing forensic pathologists in the United States and worldwide.

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#### Forensic Pathology, Deep Learning, Radiologic Imaging



### H115 Simulation-Based Learning in Forensic Medicine: The Use of 3D Photogrammetry

*Vadim Mesli, MD\**, Institut Medico Legal, Lille Cedex, Nord 59037, FRANCE; *Erwan Le Garff, MD*, Institut Médico-Légal/Forensic Institute, Lille Cedex, Nord 59037, FRANCE; *Vincent Deffaux*, Universite de Lille, Lille 59000, FRANCE; *Elodie Marchand, MD*, Universite de Lille, Lille 59000, FRANCE; *Anne A. Becart, DDS, PhD*, Service De Medecine Legale, Lille 59000, FRANCE; *Valéry C. Hedouin, MD, PhD*, Lille, Hauts De France 59037, FRANCE; *Didier Gosset, MD, PhD*, Institut de Medecine Legale, Lille 59045, FRANCE

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the potential applications of 3D photogrammetry in the field of forensic medicine teaching.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing useful insights and practical examples about 3D photogrammetry technology in a simulation-based teaching context.

Simulation-based learning in medicine concerns educational activities that use an artificial representation of a real-world medical situation. It allows the students to acquire different skills in a safe environment, contrary to in a real-life experience. This methodology is commonly used in various disciplines, such as aviation or economics. It was recently developed in a dematerialized format in the medical field but is not widely used in forensic medicine.

3D photogrammetry is a technology that makes it possible to create a 3D digital model of an object. It is based on the recording and interpretation of photographic images taken from different points of view, to obtain spatial data of the object. 3D photogrammetry was used in different fields, such as engineering, archaeology, or architecture, and was also developed in a wide variety of medical applications. The recent progress of this technology and its algorithms currently enable a large-scale use at low financial costs. This work presents potential applications of 3D photogrammetry at different stages of forensic medicine education.

The detail quality concerning the study of a body, an injury, or a pathological condition is particularly important in forensic medicine courses. Photographs are commonly used for that purpose; however, they are limited by the quality of the image, the perception of colors, the lighting, and the two-dimensional format. Video footages could also be used but are restrained in terms of interactivity with the students. The examination of real bodies could provide the best conditions, but the accessibility to the bodies for pedagogic purposes would be an issue regarding the number of students. 3D photogrammetry enables a capture of a whole organ, injury, or even a whole body in three dimensions, with good image quality. It also allows a good interactivity with the user thanks to its suitability for a touchscreen. The key features of 3D photogrammetry make it a powerful, low-cost, and user-friendly tool to improve teaching to a small or large group of students and for remote learning.

During this presentation, the use of real organs and bodies modeled in 3D with a photogrammetry software will be displayed for first, second, and third cycles of studies, post-graduation, and continuing education courses.

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### Photogrammetry, Education, Simulation



### **H116 Anatomical Distribution and Autopsy Features of Gunshot Injuries to Discriminate Between Homicides and Suicides: A Five-Year Retrospective Study From the Onondaga County Medical Examiner's Office**

*Lorenzo Gitto, MD\**, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210; *Serenella Serinelli, MD\**, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210; *Robert Stoppacher, MD*, Syracuse, NY 13210

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**Learning Overview:** After attending this presentation, attendees will better understand the most common features of fatal gunshot wounds encountered in homicides and suicides.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the most common body regions affected by gunshot wounds and the relevant autopsy features, showing their relationship with the manner of death.

Firearm-related injuries are commonly encountered by forensic pathologists worldwide. In these fatalities, a careful evaluation of all the evidence is mandatory to prevent wrong conclusions concerning the manner of death. The analysis of the location of the gunshot wounds can be useful to understand the circumstances of death. This study will provide data to support an association between a specific wound location and the manner of death.

The files of the Onondaga County Medical Examiner's Office were searched for deaths due to gunshot wounds between January 2012 and December 2016. Only cases in which gunshot wounds were the primary cause of death and in which a clear and reliable manner of death was reported were included in the study. Undetermined deaths were not considered as part of study population. The investigative report, the autopsy report, and postmortem toxicological studies were reviewed in each case. The following parameters were recorded from the files: manner of death, gender, race and age, firearms and bullets type (if available), number and sites of entrance and exit wounds, shooting distance, internal bullet pathway, blood drugs and/or alcohol concentration, and investigative narratives. The obtained data were then subcategorized as to the manner of death (suicide, homicide, and accident). The information detected on the autopsy report was used to create a graphic reconstruction of the anatomical distribution of the gunshot wounds on the body surface.

A total of 5,231 accepted cases in the studied period were found in the Onondaga County Medical Examiner's Office database, including 626 suicides, 208 homicides, and 2,275 accidents. Of these, 347 deaths due to gunshot wounds were identified and were then subcategorized based on the manner of death, showing: 238 suicides (~69%), 108 homicides (~31%), and 1 accident (<1%). A thorough discussion of the results, including graphical models showing the most common location of the injuries, and demographic data will be presented.

This study will illustrate distinct differences in suicidal and homicidal wounds that should be considered while investigating gunshot wounds related deaths. The autopsy findings, together with the scene findings, the investigative report, and the medical history of the victim, can help determine a reliable manner of death.

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#### **Gunshot Wounds, Manner of Death, Autopsy**



### **H117 The Value of Elevated Transaminases, Lipase, and Amylase in Children for Predicting Abdominal Injury After Blunt Abdominal Trauma: Is Laboratory Screening Valuable for Recognizing Physical Child Abuse? A Literature Review**

*Daniel H. Hoekstra, MD, Faculty of Medicine, University of Amsterdam, Amsterdam 1105 AZ, NETHERLANDS; Joost G. Daams, MA, Medical Library, Amsterdam University Medical Center, Amsterdam 1105 AZ, NETHERLANDS; Wouter A. Karst, MD, Department of Forensic Medicine, Netherlands Foren, The Hague 2497 GB, NETHERLANDS; Rick R. Van Rijn, PhD\*, Amsterdam University Medical Center, Amsterdam 3544MT, NETHERLANDS*

**Learning Overview:** After attending this presentation, attendees will understand the value of liver and pancreas enzyme analysis in screening cases of suspected physical child abuse.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the evidence, or lack thereof, behind liver and pancreas enzyme analysis in screening cases of suspected physical child abuse.

Recognition of abdominal injuries after blunt abdominal trauma due to physical child abuse at the emergency department can be very difficult, as the clinical history and physical examination can be deceitful. In several studies, it has been suggested that laboratory screening is useful with respect to liver and pancreas trauma.

The goal of this systematic review is to examine the predictive value of transaminases, amylase, and lipase as a screening tool for abdominal injury in cases of suspected physical abuse.

With the help of a clinical librarian, a search strategy with a focus on the predictive value of different cut-off values of transaminases, amylase, and lipase in predicting abdominal injury or liver injury, confirmed by Computed Tomography (CT), Magnetic Resonance Imaging (MRI), autopsy, or surgical exploration, was designed. Using this strategy, literature was searched in MEDLINE® and EMBASE®. Inclusion criteria were studies in which the participants were children (0–18 years old) presenting at the hospital with suspected blunt abdominal trauma due to physical child abuse or other trauma mechanisms. Studies also had to mention a cut-off value for the laboratory studies. Exclusion criteria were studies in participants older than 18 years, studies relating to penetrating abdominal trauma, and articles written in a non-western language.

A three-stage on-line selection process was used for data extraction using Covidence. Out of 4,287 papers, 15 relevant studies were identified. Data were extracted, and quality assessment was conducted with the usage of QUADAS-2. All data were summarized in an overview table. In all articles, abdominal CT scan was either the only reference test or part of a subset of reference tests. Only in two articles was it reported that the radiologists were blinded for other information when interpreting the CT scan. In the other studies in which abdominal CT scan was part of a subset of reference tests, the other reference standards were also acknowledged gold standards for this research question.

There was a significant variation in reported cut-off levels and, thus, predictive value of transaminases, amylase, and lipase in children with blunt abdominal trauma. With respect to transaminases, it was shown that were significantly higher values in cases of more severe liver injury. This was in contrast to amylase and lipase in which neither initial nor peak levels were significant predictors of a specific grade of pancreatic injury.

Limitations of the literature search were the low number of articles concerning this topic that were useful for answering the research question. Furthermore, all but two articles were retrospective or observational prospectives with an inherent higher risk of bias due to case selection or selective exclusion.

Concluding, this systematic review showed a range of cut-off values used as a threshold in laboratory screening tools for blunt abdominal trauma in children, suspected of physical child abuse, who present at the emergency department. A prospective multicenter study is needed to validate appropriate cut-off values for transaminases, amylase, and lipase. Within these, the study designs should also pay attention to the risk of radiation exposure, cost-effectiveness, timing of laboratory screening, and implementation in daily clinical practice.

#### **Child Abuse, Abdominal Trauma, Screening**



### H118 Optic Nerve Hemorrhage: A Sensitive or Specific Marker of Trauma?

Melissa M. Blessing, DO\*, Harris County Institute of Forensic Sciences, Houston, TX 77054; Merrill O. Hines III, MD, Houston, TX 77054; Dwayne A. Wolf, MD, PhD, Harris County Institute of Forensic Sciences, Houston, TX 77054; Glenn D. Sandberg, MD, Harris County Institute of Forensic Sciences, Houston, TX 77054

**Learning Overview:** After attending this presentation, attendees will: (1) better understand the optic nerve and nerve sheath anatomy, including histological differences between the Intracranial (IC) and Intraorbital (IO) components; (2) describe how to easily expose and sample the optic nerve during autopsy; (3) describe mechanisms of injury that lead to hemorrhage in one or both optic nerve compartments; and (4) determine when histological review of these components may help to elucidate the cause and manner of death in adult and pediatric autopsies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the utility of gross and histological examination of the optic nerve as it relates to the mechanism of injury and, ultimately, the cause and manner of death.

Optic nerve evaluation is a crucial component of the pediatric autopsy when abusive head trauma is suspected. Initial studies and current literature emphasize the presence or absence of Optic Nerve Sheath Hemorrhage (ONSH)—typically subdural—as an indicator of trauma.<sup>1</sup> While often associated with abusive head trauma, ONSH can be seen in non-traumatic settings and thus is non-specific.<sup>2,3</sup> Additionally, the majority of literature on this subject does not distinguish between Subdural Hemorrhage (SDH) and Subarachnoid Hemorrhage (SAH). This pilot study examines the characteristics of Optic Nerve Hemorrhage (ONH) to better elucidate IO ONH as a sensitive and/or specific marker of trauma. The IC and IO subdural potential spaces are non-contiguous; however, the subarachnoid space is connected. This pilot, therefore, also attempts to discover the frequency of SAH extension between the IC and IO compartments and elucidate the role of Cerebrospinal Fluid (CSF) flow in the movement of SAH under traumatic and non-traumatic circumstances.

Medical examiner cases with gross evidence of SAH with and without SDH were collected over the one-month pilot portion of this ongoing study. Bilateral segments of IC optic nerve were sampled anterior to the optic chiasm. The orbits were unroofed using an autopsy saw along the medial, lateral, and anterior margins of the anterior cranial fossa followed by removal of cut bone and orbital periosteum. The exposed IO portion of the optic nerves was sampled without removal of the globe. Cases with grossly evident ocular injuries were excluded. All specimens were fixed in formalin, processed in the usual manner, and evaluated with hematoxylin and eosin. The presence or absence of gross and microscopic SDH and SAH was recorded and reviewed in conjunction with autopsy photographs and reports.

Nine cases with SAH hemorrhage were collected during the one-month period; four had concurrent SDH. Ages ranged from 16 to 84 years (median: 53; mean 45); 6 (67%) were men. The majority of cases ( $n=8$ ; 89%) involved trauma. Causes of death included gunshot wound(s) of the head ( $n=5$ ; 56%), multiple blunt force injuries ( $n=3$ ; 33%), and ruptured cerebral aneurysm ( $n=1$ ; 11%). Most traumatic cases had skull fractures ( $n=7$ ; 78%); of those, 5 (71%) involved the orbit. Of the nine cases, only two demonstrated SAH in both the IC and the IO compartments: one trauma case with orbital fractures, and the ruptured cerebral aneurysm. No correlation between IC and IO SDH was seen.

Extension of SAH from the IC to IO compartments under non-traumatic circumstances is likely due to the high volume and arterial pressure, such as in the case of aneurysm rupture. Extension of SAH from the IC to IO compartments under traumatic circumstances is rare; primary optic nerve injury, including from abusive head trauma, is a more likely mechanism of IO ONH. These findings will be expanded upon as the study continues and incorporates a greater number of infant and pediatric cases.

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#### Forensic Neuropathology, Optic Nerve Hemorrhage, Trauma





### H119 The Correlation of Pediatric Cervical Nerve Root and Dorsal Root Ganglia Hemorrhage With Hemorrhage at Other Spinal Cord Levels

Marianne E. Beynon, MD\*, Harris County Institute of Forensic Sciences, Houston, TX 77054; Miriam E. Soto Martinez, PhD, Harris County Institute of Forensic Sciences, Houston, TX 77054; Jo Elle G. Peterson, Oklahoma City, OK 73104; Si Gao, MS, Harris County Institute of Forensic Sciences, Houston, TX 77054; Jennifer C. Love, PhD, Office of the Chief Medical Examiner, Washington, DC 20024; Dwayne A. Wolf, MD, PhD, Harris County Institute of Forensic Sciences, Houston, TX 77054; Glenn D. Sandberg, MD, Harris County Institute of Forensic Sciences, Houston, TX 77054

**Learning Overview:** After attending this presentation, attendees will be: (1) familiar with a standardized, concise methodology for evaluating and reporting Nerve Root and Dorsal Root Ganglia (NR/DRG) hemorrhage in the pediatric autopsy, and (2) knowledgeable regarding the correlation between pediatric cervical NR/DRG hemorrhage and NR/DRG hemorrhage at other spinal cord levels.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information regarding the correlation between pediatric cervical NR/DRG hemorrhage and NR/DRG hemorrhage at other spinal cord levels, thereby assisting forensic pathologists and neuropathologists in performing and interpreting the pediatric autopsy.

Evaluation of cervical NR/DRG hemorrhage has become increasingly recognized as a crucial component of the pediatric autopsy by the forensic pathology community since an association between this hemorrhage and pediatric Traumatic Head Injury (THI) was first described in 2005.<sup>1</sup> Matshes et al. postulates that these hemorrhages occur secondary to forces transferred to the NR/DRG during hyperflexion-extension of the infant neck.<sup>2</sup> They further suggest that involvement of C3–C5 nerve roots would lead to diaphragmatic paralysis with subsequent anoxic brain injury.<sup>2</sup> Additional research has supported the association between cervical NR/DRG hemorrhage and pediatric THI, including studies at the Harris County Institute of Forensic Sciences (HCIFS).<sup>3–7</sup> As part of the comprehensive investigation of NR/DRG hemorrhage performed by the HCIFS, a comparison between cervical NR/DRG hemorrhage and NR/DRG hemorrhage at other spinal cord levels was performed.

A nine-month prospective study was conducted, as previously described.<sup>7</sup> All infants, 0–12 months old at time of death, autopsied by the HCIFS were included, excepting individuals with survival time greater than one week after terminal hospital admission. The spinal cord with NR/DRG at all levels was removed via modified posterior approach.<sup>6</sup> The tissue was fixed for two weeks in 20% formalin, then sectioned and Hematoxylin-Eosin (H&E) stained following standard methods. Each section was examined by two persons who were blinded to cause and manner of death. Hemorrhage in each NR/DRG was scored on a scale (0=no hemorrhage, 1=scant, 2=prominent).<sup>7</sup> The scores were standardized to facilitate direct comparison between spinal cord levels.

Over the study period, 62 cases were collected. Sixty infants from birth to 11 months (median age 3.5 months) were included in the study. One case was excluded from the study because the cause of retinal and NR/DRG hemorrhage could not be definitively attributed to (or excluded from) trauma, and one due to administrative error. Forty-nine infants died from natural, non-traumatic causes (non-trauma group) and 11 from traumatic injury (THI; undetermined or homicidal manner; trauma group). The number of NR/DRG recovered from each case varied. Cervical and thoracic sections were present in all 60 cases, lumbar in 57 cases, sacral in 55 cases, and cauda equina in 53 cases. Overall, recovery of thoracic through lumbar NR/DRG (94% and 84%, respectively) was similar to the cervical region. On average, 6 cervical, 4 thoracic, and 1 each of lumbar, sacral, and cauda equina spinal cord levels were examined in each case.

Overall, NR/DRG hemorrhage at the other four spinal cord levels showed good correlation with that of the cervical region. In the non-trauma group, 85.7% of both cervical and non-cervical NR were without hemorrhage, and hemorrhage was absent in 95.9% of cervical DRG and 91.8% of non-cervical DRG. In the trauma group, 90% of cases with cervical NR hemorrhage and 100% of cases with cervical DRG hemorrhage showed hemorrhage at the other levels. All discrepancies between cervical and non-cervical hemorrhage involved rare scores of 1 (sparse hemorrhage), with poor agreement between the two observers, consistent with previously reported data.<sup>7</sup> In addition, a statistically significant difference in NR/DRG hemorrhage severity between the trauma and non-trauma groups was seen at each spinal cord level ( $p < 0.01$ ), as well as the spinal cord in its entirety ( $p < 0.001$ ).

To conclude, these data suggest that forces imparted during trauma are not limited to the cervical spine but may cause significant damage to all levels of the pediatric spinal cord. If Matshes' theory linking cervical trauma and diaphragmatic paralysis is extrapolated to the non-cervical cord, many different body processes may suffer detrimental effects, including intercostal muscle dysfunction, which would further compound respiratory compromise.<sup>2</sup> In addition, non-cervical NR/DRG hemorrhage shows similar promise to its cervical counterpart in distinguishing traumatic and non-traumatic deaths in the pediatric population.

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### **Cervical Spinal Cord, Nerve Root, Dorsal Root Ganglia Hemorrhage**



### H120 Ventriculoperitoneal Shunt Occlusion Resulting in Acute Hydrocephalus and Death in Developmentally Delayed Patients: Two Case Reports

Joshua White, BS\*, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49007; Joseph A. Prahlow, MD, Western Michigan University School of Medicine, Kalamazoo, MI 49007

**Learning Overview:** After attending this presentation, attendees will be familiar with the indications for and complications of Ventriculoperitoneal (VP) shunts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting two cases of death related to acute hydrocephalus related to VP shunt occlusion occurring in chronically disabled patients with hydrocephalus.

The standard clinical strategy for managing conditions with chronic hydrocephalus involves the placement of a VP shunt. Although the successful function of VP shunts has improved since their initial implementation, VP shunt failure remains a persistent issue of concern, with approximately half of all VP shunt patients requiring replacement or revision of their shunt.<sup>1,2</sup> One common cause of shunt failure involves the physical obstruction of the device, either by biological tissue or organic material.<sup>1</sup> Although death related to obstruction of VP shunts is a recognized complication, the mortality rate related to VP shunt obstruction is poorly defined. This study describes two cases of death related to acute hydrocephalus resulting from obstruction of VP shunts.

**Case 1:** A 23-year-old female cerebral palsy patient, with additional history of congenital hydrocephalus, mental handicap, a VP shunt, and seizures, was admitted to a hospital for complaints of abdominal pain, nausea, and vomiting of three days duration. She received supportive care for a diagnosis of “gastroenteritis” and was discharged to home in a markedly improved condition. During hospitalization, she had no known neurologic complaints; however, three days after discharge, she was found unresponsive and apneic at her group home. Despite initial successful resuscitation, she was pronounced braindead shortly after admission. A limited, head-only, hospital autopsy was performed at the request of the legal next-of-kin.

Autopsy disclosed diffuse cerebral edema with associated cerebellar tonsillar and cerebral uncal herniation. Evaluation of the VP shunt revealed proximal occlusion by a soft, red-tan substance, which, on microscopic exam, was composed of fibrovascular tissue with chronic inflammatory cells. The cause of death was ruled as complications of hydrocephalus/cerebral palsy, with proximal occlusion of VP shunt.

**Case 2:** A 15-year-old cerebral palsy patient, with additional history of meningomyelocele, seizures, and hydrocephalus with VP shunt, presented to an Emergency Department (ED) with complaints of progressively worsening headache and neck pain. Upon arrival at the ED, he experienced a seizure and became unresponsive. He was resuscitated, and an emergent computerized tomography scan of the head showed increased size of the 3<sup>rd</sup> and lateral ventricles, compared to previous studies. Despite emergent decompression, he died the next day. An autopsy was performed at the request of the legal next-of-kin.

Autopsy revealed marked cerebral edema with associated cerebellar tonsillar herniation and hemorrhage, adjacent medullary hemorrhage, and dilated ventricles. The VP shunt had numerous areas of intraluminal precipitate and dilations along its course but was patent except for a complete obstruction just inferior to the valve and reservoir device, behind the patient’s ear. The cause of death was ruled as acute obstructive hydrocephalus due to VP shunt obstruction related to underlying meningomyelocele and cerebral palsy.

Although VP shunt failure is relatively common, death related to such failure is relatively rare, or at least not well-described in the literature. A 2004 study of pediatric patients in five regional hospitals over a ten-year period reported only eight deaths related to shunt failure.<sup>3</sup> A search for similar studies in older patients could not be found. The presented cases serve to highlight the importance of shunt evaluation at autopsy in cases of sudden, unexpected death in this vulnerable patient population.

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#### Ventriculoperitoneal Shunt, Hydrocephalus, Death



### H121 Biochemical and Histopathological Findings in Sudden Unexpected Death in Epilepsy

*Shyam Kishore, MD, Forensic Medicine, All India Institute of Medical Science, New Delhi 110029, INDIA; Chittaranjan Behera, MD\*, Forensic Medicine, All India Institute of Medical Science, New Delhi 110029, INDIA; Sudhir K. Gupta, MD, All India Institute of Medical Science, New Delhi 110029, INDIA; Sudheer Arava, MD, Pathology, All India Institute of Medical Science, New Delhi 110029, INDIA; Asit Ranjan Mridha, MD, All India Institute of Medical Science, New Delhi 11029, INDIA; Ashok K. Jaiswal, PhD, Forensic Medicine, All India Institute of Medical Science, New Delhi 110029, INDIA*

**Learning Overview:** After attending this presentation, attendees will understand the biochemical and histopathological changes in the body of the deceased in cases of sudden and unwitnessed death in epilepsy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing various biochemical and histopathological changes occurring in various organs in cases of epileptic death and deciding the cause of death when the epilepsy seizure at the time of death is not witnessed.

One of the causes of death in epilepsy is the Sudden Unexpected Death in Epilepsy (SUDEP). It can occur without being in a status epilepticus or even without a typical seizure. It is estimated to cause nearly 2,000 deaths per year in the United States alone and accounts for as many as 15% of all epilepsy-related deaths. The most consistent risk factor across multiple studies is an increased frequency of Generalized Tonic-Clonic Seizures (GTCS). In a developing country like India, with a huge burden of epileptic patients, sudden unwitnessed deaths are on the rise. These deaths are deemed to become a medicolegal case and autopsy are warranted to establish the cause of death.

Based on these facts, this study was undertaken to have a comprehensive examination of each case of sudden death in epilepsy, including gross findings, histopathology, toxicological, and biochemical analysis. Toxicological screening for alcohol and common antiepileptic drugs was conducted on the femoral blood sample. Biochemical analyses for electrolytes, viz, sodium, potassium, calcium, glucose, and cardiac biomarkers (CPK-MB, CPK-NAC) was done on femoral blood, vitreous, Cerebrospinal Fluid (CSF), and pericardial fluid. The findings were compared with those of non-epileptic cases. Sections for histopathology were obtained from different areas of the brain (cerebral cortex, cerebellum, hippocampus, and any grossly visible pathological lesions), lung (hilar and peripheral region, and any grossly visible pathological lesions), and heart (coronary artery, myocardium any grossly visible pathological lesions) in epileptic and non-epileptic deaths.

Most of the cases of epilepsy were of the generalized tonic-clonic variety and some cases were of partial seizure. Duration of disease was between 1 to 12 years. A history of regular medication use was seen in only a few cases; most were either taking the medicine irregularly or not at all. Seizure attack at the time of death was seen in only some cases and most of the deaths were unwitnessed. No history of epilepsy was found in non-epileptic deaths.

On external examination of epilepsy cases, tongue bite, gingival hyperplasia, and features of aspiration were seen. Internally, non-specific congestion of the internal organs was present. Pulmonary edema was found in all cases of either mild/moderate/marked and coronary artery atherosclerosis. Among the non-epileptic cases, external examination show ligature marks, diffuse burn injury, and electrocution marks. Internally, pulmonary consolidation, subarachnoid hemorrhages, and non-specific organ congestion was seen, which were the cause of death in respective cases.

This study has found a lower potassium level, higher sodium level, and higher CK-NAC and CK-MB levels among epileptic deaths in comparison to non-epileptic deaths. There was no significant difference in the level of calcium and glucose between the two groups. On histopathology of various organs in epileptic deaths, the cerebral cortex shows ischemic changes in five cases, meningeal inflammation in one case, inflammation in the cerebellum in one case, and ischemia and calcification of the hippocampus in one case. The findings in the sections of the lung were pulmonary edema of various grades, pulmonary congestion in 12 cases, bacterial colonization in two cases vegetative matter in two cases and autolysis in one case. Sections of coronary showed fibro-atheromatous plaque in four cases and ischemic changes and replacement fibrosis in five cases. Histopathological examination in non-epileptic cases show no significant findings in the cerebral cortex, cerebellum, hippocampus, the sections of lung, coronary artery, and the myocardium in most of the cases, except fibro-atheromatous plaque in four cases, and pulmonary consolidation in one case.

This study concludes that biochemical and histopathology of the organs contain some specific changes in epileptic deaths, such as lower potassium levels, higher sodium, CPK-MB and CPK-NAC levels, ischemic changes of the cerebral cortex, inflammation of the meninges and the cerebellum, calcification of hippocampus, and focal replacement fibrosis of the myocardium.

#### Sudden Death in Epilepsy, Cause of Death, Biochemical Marker



### H122 Evaluation of the Role of Polymerase Chain Reaction (PCR) -Based Postmortem Respiratory Infectious Disease Panels in Infant Death Investigations

Abigail Jacqueline Grande, MPH\*, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49008; Joyce L. deJong, DO, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49008; Amanda O. Fisher-Hubbard, MD, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49007

**Learning Overview:** After attending this presentation, attendees will better understand the difficulties in identifying the role of infectious diseases in apparent Sudden Unexplained Infant Deaths (SUIDs).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining the contribution from microbiological testing to the investigation of infant deaths.

In 2016, there were 23,161 infant deaths (under one year of age) in the United States, of which most were attributed to congenital malformations, low birth weight, and SUIDs.<sup>1</sup> Although the utility of postmortem microbiology in infant deaths is frequently debated, it is essential in understanding the factors that may have contributed to the death of an infant.<sup>2</sup> Many respiratory pathogens are potentially fatal, including Respiratory Syncytial Virus (RSV), which is the most common cause of bronchiolitis and pneumonia in children under one year in the United States.<sup>3,4</sup> Previous studies examining the role of respiratory infections in infant deaths have found that rhinovirus/enterovirus and RSV were the most commonly detected pathogens, with 60% of cases testing positive for RSV actually contributing to the cause of death.<sup>3</sup> No single respiratory infection has been causally linked to SUIDs, but many studies have demonstrated higher rates of respiratory pathogens isolated from SUID cases in comparison to controls.<sup>5</sup>

For this study, respiratory infectious disease panel results were reviewed. This testing uses a multiplexed nucleic acid test using a FilmArray instrument for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs. This testing includes the detection of: adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus NL63, human metapneumovirus, human rhinovirus (1, 2, 3, and 4), enterovirus, influenza A (H1-2009, H1 and H3), influenza B, parainfluenza (1, 2, 3, and 4), respiratory syncytial virus (RSV), *Bordetella pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*. *Bordetella parapertussis* was added to the respiratory infectious disease panel in late 2017. Of note, rhinovirus and enterovirus are of the same genetic family and cannot be definitively distinguished using molecular testing.

This study reviewed 100 infant deaths investigated by medical examiners and/or coroners from multiple counties in Michigan and Indiana from 2014 to 2018. This study was restricted to infants, defined as age zero to one year for a more specific analysis. Of these 100 infant deaths, a respiratory infectious disease panel was performed on a nasopharyngeal swab in 70 cases. Of these 70 cases, 39 infants (56%) tested positive for one or more respiratory pathogens. A total of 53 respiratory pathogens were detected among these 39 infants; nine infants tested positive for more than one respiratory pathogen. Of the 39 infants testing positive for a respiratory pathogen, 24 infants (61%) tested positive only for rhinovirus/enterovirus, with another 9 infants testing positive for one or more of pathogens that included rhinovirus/enterovirus, for a combined total of 33 infants (85%) testing positive for rhinovirus/enterovirus. Adenovirus was positive in 5 cases; coronavirus OC43 and parainfluenza 3 were positive in 3 cases each; influenza B and RSV were positive in 2 cases each; and coronavirus HKU1, metapneumovirus, influenza A, parainfluenza 1, and *Mycoplasma pneumoniae* were positive in 1 case each.

Of the 39 cases with positive respiratory infectious disease panels, the forensic pathologist reported a respiratory infection as a contributing factor to the death in only 16 (41%) of the deaths. Even with a contributing respiratory infection, 13 (81%) of these deaths were still certified as indeterminate due to other possible contributing factors, such as unsafe sleeping conditions.

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#### Infant Deaths, Microbiology, Respiratory Infections



### H123 Severe Pulmonary Artery Hypertension Persisting After a Late Atrial Septal Defect (ASD) Closure: Presentation of a Case

*Serenella Serinelli, MD\**, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210; *Lorenzo Gitto, MD\**, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210

**Learning Overview:** After attending this presentation, attendees will understand the pathophysiology and the autopsy findings of pulmonary artery hypertension after a late ASD closure.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that pulmonary hypertension can persist after a late surgical repair of ASD.

ASD is the most prevalent congenital cardiac anomaly in adults, representing ~35% of all congenital heart defects. It consists of a heart defect in which blood flows between the atria of the heart: oxygen-rich blood can flow directly from the left side of the heart to mix with the oxygen-poor blood in the right side of the heart, or vice versa. This can lead to lower-than-normal oxygen levels in the arterial blood that supplies organs and tissues. Due to the high blood flow from left to right, an ASD can also cause right ventricular remodeling and failure, with enlargement of right cardiac chambers, severe arrhythmias, and pulmonary artery hypertension. The left ventricle can be smaller than the right one and a reduced coronary perfusion can occur.

Most adults with ASD are safely treated using available techniques of surgical or percutaneous repair and become free of complications over the long term. However, there is a small fraction of patients who are at risk for post-operative complications, particularly the persistence of elevated pulmonary vascular resistance and pulmonary artery hypertension.

This presentation describes the case of a 47-year-old Asian female (height: 152cm; weight: 36kg) who was found unresponsive by her husband in the bathroom of their house. The husband promptly called the emergency medical services. A cardiopulmonary resuscitation was performed with negative results and the woman was pronounced dead.

According to the medical records, the woman underwent a surgical repair of an atrial septal defect at the age of 37 and suffered from severe pulmonary hypertension, high blood pressure, paroxysmal atrial fibrillation, and multivalvular insufficiency. A few months prior to death, she had an episode of acute heart failure and underwent a chest-abdomen Computed Tomography (CT) and an echocardiogram that showed: persistence of a left superior vena cava probably draining in the coronary sinus, enlargement of the right heart with massive tricuspid regurgitation, enlargement of the pulmonary arteries with severe pulmonary regurgitation, bilateral pleural effusion, atelectasis of the left lung, chronic passive congestion of the liver, and ascites.

At the autopsy, the heart weighed 650 grams and showed: an enlargement of the coronary sinus, a correctly positioned interatrial patch, a notable right ventricular hypertrophy with a small left ventricle, enlarged pulmonary arteries with fatty streaks, and pulmonary and tricuspid valve insufficiency. It was not possible to identify the left superior vena cava detected during the imaging studies; this could be due to the relatively small size of the vessel or to the presence of massive adhesions from prior surgery.

At the microscopic examination, the heart showed multiple myocardiocytes with enlarged nuclei and multiple areas of fibrosis. The lung showed hypertrophy of the media of the arterioles and plexiform lesions, findings consistent with a severe pulmonary artery hypertension.

In this case, since the woman did not have the defect closed until such an advanced age (37 years old), probably all the autopsic findings could be attributed to the ASD. The left superior vena cava persistence may have contributed. A prolonged flow from the left atrium across the ASD to the right atrium caused the right ventricular hypertrophy and pulmonary hypertension. Moreover, the left ventricle was reduced in size and a myocardial fibrosis (presumably due to low coronary perfusion) was observed. It was not possible to understand if, in this case, the delay in surgical treatment was due to a late presentation of the defect or due to different reasons. In any case, the late surgical treatment allowed the subject to become free from the interatrial communication, but not from the advanced right ventricular remodeling and pulmonary vasculopathy, which caused death.

#### Atrial Septal Defect, Pulmonary Hypertension, Heart Remodeling



### H124 Chronic Traumatic Encephalopathy Pathology Following Shotgun Injury to the Brain

Rudy J. Castellani, MD\*, Center for Neuropathology, Kalamazoo, MI 49008; Taylor Tribett, BS, Western Michigan University School of Medicine, Kalamazoo, MI 49007; Theodore T. Brown, MD, Kalamazoo, MI 49008

**Learning Overview:** After attending this presentation, attendees will: (1) assess significance of tau pathology after traumatic brain injury, (2) correlate tau pathology with clinical signs, (3) interpret consensus guidelines for Chronic Traumatic Encephalopathy (CTE) interpretation, and (4) assess appropriateness of the CTE diagnosis for death certification

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by clarifying broad misunderstanding regarding brain interpretation for CTE pathology, its significance in terms of clinical problems during life, and the lack of appropriateness of the CTE diagnosis for death certification

CTE was suggested initially in boxers (i.e., dementia pugilistica) who developed neurological abnormalities after long boxing careers. Autopsy studies in the mid-20<sup>th</sup> century variably highlighted Neurofibrillary Tangles (NFT) among the features of dementia pugilistica, prompting the hypothesis that dementia pugilistica shares pathogenic similarities with Alzheimer's Disease (AD). In the past decade, the CTE concept has extended to other athletes, although with limited clinical correlations and more subtle neuropathology. A consensus group has recently set provisional criteria for CTE pathology and highlighted a pattern of phosphorylated tau (p-tau) neuropathology considered "pathognomonic" for CTE. The consensus group did not adopt a staging scheme for CTE and did not address the question of whether CTE pathology represents the substrate for a progressive neurodegenerative disease.

Reported here is the case of a 63-year-old man who suffered a shotgun injury to the brain in his early twenties. He remained neurologically compromised for 42 years after the injury but showed no evidence of neurological disease progression after the initial injury. He had no other Traumatic Brain Injury (TBI) exposure and did not play football or other high-energy collision sports. Neuropathological examination confirmed tissue damage from the shotgun injury, with multiple foci of encephalomalacia. Also present were mammillary body atrophy, atrophy of the fornices, and an atrophic and convex thalamus, gross features occasionally described in historical cases of dementia pugilistica. Mammillary body atrophy and evidence of previous TBI are listed as supportive neuropathological features of CTE. Also present were localized deposits of p-tau within neurons and astrocytes around small blood vessels at the depths of cortical sulci, meeting neuropathological criteria for CTE. P-tau and TDP-43 deposits within marginal brain tissue damaged by penetrating shotgun injury were also present focally, indicating focal proteinopathy as a direct consequence of neurotrauma. No amyloid- $\beta$  (A $\beta$ ) deposits were present.

These findings indicate that CTE pathology may occur in the aftermath of single, severe TBI. The fact that it remained stationary for decades with no evidence of clinical or pathological progression, and no evidence of AD pathology, raises questions about the TBI-progressive proteinopathy paradigm, and the theory that CTE pathology is a hallmark for neurodegenerative disease. The absence of correlation between p-tau pathology in this case and in the CTE literature in general, along with the lack of disease progression in a manner of canonical neurodegenerative disease, suggests that CTE is a descriptive neuropathological, and at times a purely immunohistochemical entity, that lacks clinical meaning. Moreover, an abundance of literature calls into question a cause-effect relationship between TBI and progressive neurodegenerative proteinopathy. The many uncertainties surrounding the CTE concept suggests that CTE is inappropriate for death certification and may lead to unnecessary confusion. More research is needed before concluding that CTE is a cohesive clinicopathological entity responsible for neurological or psychiatric problems during life.

#### Traumatic Brain Injury, Chronic Traumatic Encephalopathy, Tauopathy



### **H125 Massive Intracranial Bleeding Due to the Rupture of a Rare Spontaneous Pseudoaneurysm of the Middle Cerebral Artery in a Pediatric Patient: A Case Report With Clinical, Radiological, Gross, and Microscopic Findings**

*Lorenzo Gitto, MD\*, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210; Serenella Serinelli, MD\*, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210*

**Learning Overview:** After attending this presentation, attendees will understand the features and appearance of a rare intracranial idiopathic pseudoaneurysm.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the features of a rare cause of intracranial bleeding in a pediatric patient.

Pediatric pseudoaneurysms are a rare and underdiagnosed cause of intracranial hemorrhage. Regarding the head and neck districts, approximately 80% of the pseudoaneurysms affect extracranial vessels (mostly carotid and vertebral arteries), while only 20% are intracranial. The most common etiologies of pseudoaneurysm are trauma, infection, drugs, or neoplasm. Spontaneous/idiopathic pseudoaneurysms are rarely observed in the clinical and forensic practice.

A 14-year-old White female was brought to the hospital after she lost her consciousness while attending her morning school session. She had no pertinent familial history or history of traumas. According to her recent medical history, she had a heavy menstrual bleeding, with a three-week-long recurrent mild headache, and a lack of appetite started the night before. On arrival at the emergency department, the neurological examination showed a drowsy but easily arousable girl, with a Glasgow Coma Scale (GCS) score of 13 (E3V4M6). Physical and neurological examinations were unremarkable. A pregnancy test was negative. An Electrocardiogram (EKG) showed no cardiac rhythm alterations. Plain computed tomography of the brain without contrast showed an intra-axial hemorrhage in the right temporal lobe, 6cm in diameter, without evidence of skull fractures; it produced a mass effect, without deviation of the median line structures. Cerebral edema and subarachnoid hemorrhage were also noted. After the neuroradiological study, the GCS score decreased to 10 (E2V3M5).

The patient was referred to the Neurosurgery Department of a primary health care provider. Upon arrival, the patient underwent a plain computed tomography of the brain with contrast that showed an increase of the intraparenchymal hematoma in the right cerebral hemisphere with a 10-centimeter dislocation of the median line structures. The patient immediately underwent a decompressive craniotomy, and an external ventricular drainage was placed. A cerebral angiography was performed, showing an active bleeding from a right middle cerebral artery pseudoaneurysm, and an endovascular aneurysm repair was performed.

Despite the multiple neurosurgical operations, the patient died after three days of coma due to the massive intracranial bleeding.

At the postmortem examination, the young girl was an organ donor, but the whole brain was saved in formalin for a neuropathology consultation. At the examination of the fixed brain, a massive subarachnoid hemorrhage was observed on the right cerebral hemisphere, together with an extensive right temporal lobectomy as a result of the neurosurgical intervention. Following the right branches of the circle of Willis, the right middle cerebral artery was found: it showed the presence of a fusiform vessel dilatation, 2.5x1 centimeters in size, filled by surgical glue.

The microscopic examination of the vessel dilatation confirmed a pseudoaneurysm of the right middle cerebral artery, showing the loss of normal arterial architecture with intermittent disruption of the internal elastic lamina and vacuolar degeneration of the tunica media.

Unlike true aneurysms, pseudoaneurysms are typically found along a vessel wall, distal from a branch point. They differ pathologically from true or dissecting aneurysms. In pseudoaneurysms, there is a disruption of all three layers of the vessel wall with a contained hematoma. They have a specific wall formed by hematoma organization and fibrosis with the surrounding and connective tissue indicating vessel wall injury. Pseudoaneurysms must be distinguished from true saccular aneurysms because of their friable nature, the tendency of rupture, and different pathologic findings.

At the postmortem examination, a massive intracranial hemorrhage or a previous surgical manipulation of the brain may hide the existence of a vessel malformation. Therefore, in the case of pediatric intracranial bleeding, a multidisciplinary approach (clinicians, radiologists, neurosurgeon, pathologists, and neuropathologists) is required, and a careful inspection of the central nervous system structures is mandatory.

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#### **Pseudoaneurysm, Intracranial Bleeding, Pediatrics**





### H126 Dorsal Root Ganglia and Nerve Root Hemorrhage in Resuscitated Respiratory Syncytial Virus (RSV) Bronchiolitis

Nathan S. Shaller, MD\*, Wake Forest Baptist Medical Center, Winston Salem, NC 27157; Anna G. McDonald, MD, Wake Forest Baptist Medical Center, Winston Salem, NC 27157

**Learning Overview:** The goal of this presentation is to show the ischemia-reperfusion injuries of the central nervous system in resuscitated RSV bronchiolitis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by furthering the understanding of ischemia-reperfusion injury once thought to be specific for trauma.

Autopsy findings in pediatric deaths can be challenging due to the complex, evolving, and occasionally conflicting body of literature that obfuscates the distinction between pathologic findings and artifacts of resuscitation. This presentation reports two autopsy cases of RSV in children with secondary bronchopneumonia and cardiopulmonary arrest that highlight the importance of autopsy, including fundal, brain, spinal cord, spinal nerve root, and dorsal root ganglia examination.

**Case 1:** A 5-month-old girl without significant past medical history was brought by her mother to the Emergency Department (ED) with a one-day history of fever (measured at home at 101°F), cough, and congestion. She was diagnosed with a viral upper respiratory illness, advised to take acetaminophen, and sent home. The girl was brought to the ED later the same evening with decreased intake and multiple episodes of emesis with continued fever (recorded in ED at 103.2°F). She was given ondansetron and discharged home. Two days later, at approximately 4:30 a.m., the father found the infant unresponsive and facedown on the couch. She was brought to the ED pulseless and apneic. Despite maximal cardiopulmonary resuscitative efforts, there was no return of spontaneous circulation.

**Case 2:** A 9-month-old girl with a history of sickle cell trait was brought to the ED for congestion, cough, and fever. A RSV rapid antigen test was negative. A chest radiograph showed clear lung fields throughout. She was diagnosed with an upper respiratory tract infection and discharged home with prednisolone and amoxicillin. The following day, the infant was being transported to the ED by her father when she suffered cardiopulmonary arrest in the father's vehicle. Emergency services were contacted and transported the infant to the ED. She arrived apneic and without palpable pulses. She eventually had return of circulation after approximately 30 to 40 minutes of downtime. A chest X-ray showed a suspicion for multi lobe airspace disease. She was subsequently transferred to Wake Forest Baptist Medical Center Emergency Department where her pupils were noted to be fixed and dilated on arrival. That evening, the decision was made to transfer her care to comfort measures. Late that evening, she had loss of pulses for approximately two minutes with return of circulation. Ophthalmology noted bilateral retinal hemorrhages at this time. Her clinical status deteriorated, and she was pronounced approximately 12 hours after admission.

Autopsy findings for both cases identified RSV genetic material on postmortem nasopharyngeal swab, as well as lungs with bronchiolitis and necrosis of respiratory epithelium. Secondary bacterial bronchopneumonia was also present on microscopic sections in both cases with *Lactococcus lactis* isolates recovered from lung and blood cultures in Case 1. Postmortem indirect ophthalmologic examination confirmed bilateral retinal hemorrhages in Case 2. Spinal cord and dorsal root ganglia were examined in both cases. Case 1 showed no ischemic neurons in the brain sections examined as well as spinal cord and dorsal root ganglia without significant hemorrhage, necrosis, or inflammation. In contrast, examination of the cord in Case 2 showed diffuse hypoxic-ischemic changes of the neurons within the anterior and posterior horns. Dorsal root ganglia from C6/7 showed hemorrhage along the fascicles of the nerve roots with superficial extension into the dorsal root ganglia. Intradural hemorrhage was prominent around lumbar nerve roots. Additionally, Case 2 showed an enlarged spleen with diffusely enlarged red pulp. Sickled red blood cells were identified throughout microscopically examined tissue. These findings suggest an element of red cell sequestration due to sickling in the setting of hypoxic stress of RSV bronchiolitis and secondary bronchopneumonia.

Diffuse hypoxic-ischemic injury of the brain and spinal cord is a non-specific finding in pediatric deaths. Dorsal root ganglial hemorrhage has previously been reported to be mechanistically associated with hyperextension/hyperflexion neck injury in young children. This study proposes that the dorsal root ganglia and spinal cord hemorrhage may reflect ischemia-reperfusion injury in the setting of prolonged resuscitation with return of circulation. These cases highlight the importance of routine spinal cord examination in the pediatric autopsy to further our understanding of reperfusion injury in the central nervous system previously thought to be specific for trauma.

#### Resuscitation, Ganglia, Hemorrhage



### H127 An Evaluation of Cardiac Implantable Electronic Devices in Forensic Practice: How It Can Improve the Medicolegal Analysis of Cause of Death in Postmortem Analysis and Even Answer Unexpected Questions in the Living

*Justine Canales, MD\*, Institut Medico Legal, Tours 37044, FRANCE; Camille Rerolle, MD, Service de Medecine Legale, Tours 37000, FRANCE; Pauline Saint-Martin, MD, PhD, Service de Medecine Legale, Tours 37000, FRANCE*

**Learning Overview:** After attending this presentation, attendees will better understand various situations for which the analysis of a pacemaker or a defibrillator device were useful in forensic practice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting several case reports in which the pacemaker or defibrillator interrogation provided essential information to the forensic pathologist.

**Introduction:** Cardiac Implantable Electronic Devices (CIEDs) are commonly implanted in patients suffering from various heart conditions. They encompass Pacemakers (PM) and Implantable Cardioverter Defibrillators (ICDs). Most of the devices have an automatic cardiac rhythm recording feature, allowing it to be interrogated at any moment to provide important information for the patient's follow-up; but interrogation can also be made in a forensic context. Presented here are three cases in which analysis of CIEDs was useful in a routine manner.

**Case 1:** A 30-year-old male was found dead at home. He had attended a party the day before. The deceased had a history of third-degree atrioventricular block. He had a PM since the age of 20. During the autopsy, the cause of death could not be specified. Toxicology and histology were still pending while this abstract was being written. The PM analysis revealed that at 9:18 a.m. the morning of his death, a ventricular tachycardia followed by several minutes of ventricular fibrillation were recorded that did not allow survival. The estimated time of death given by the physician who delivered the death certificate was around 2:30 p.m. the same day. That was not consistent with the device data analysis.

**Case 2:** An 86-year-old male suffered from terminal heart failure. He had a history of ventricular arrhythmia and carried an ICD. He was brought to the emergency room because he had signs of acute decompensated heart failure. His family, who had recently decided with his physician to limit any invasive medical intervention, was not consulted and the patient was transferred to several departments before he died in the intensive care unit, 48 hours after his admission. During the autopsy, signs of severe cardiac heart failure were present and confirmed by histology. Toxicology showed a lethal concentration of digoxine, which was part of the regular treatment of the deceased. The question of a digoxin poisoning that could be partly due to error in prescription and/or in medication administration was asked by the family. First, the ICD analysis showed that the device ran properly. Second, no arrhythmic episode could be detected during the two days of hospitalization. As a high concentration of digoxin is not interpretable by itself if not correlated to cardiac arrhythmia, it was concluded that the cause of death could not be directly related to a digoxin poisoning but was due to terminal heart failure.

**Case 3:** A 45-year-old male, suffering from a Brugada syndrome with a mixed anxiety-depressive disorder, reported being the victim of verbal and physical assault within a six-month period by two individuals. As the physical violence was particularly severe, investigators wanted to know whether the ICD he was carrying for the cardiac condition had registered any abnormal activity and had delivered any electrical shock during the time of his sequestration. The patient's ICD was analyzed with his consent at the request of the investigators. The analysis showed that no abnormal cardiac activity had been recorded within the past two years prior to the complaint.

**Discussion:** The importance of CIED interrogation in a forensic context has already been described in the literature. This analysis can answer questions regarding the circumstances and the cause and manner of death. The first case was classic and provided an example of determining the last event before death. The second case was more unusual and contributed to the analysis of a medical malpractice case. Finally, the last case showed that CIEDs may also be interesting for a case with a living person. These three cases confirm forensic institutes should proceed with a systematic CIED interrogation for all individuals carrying these devices as they can provide important additional information to the medicolegal investigation.

**Forensic Science, Forensic Pathology, Pacemaker**



### H128 A Comparison of Coronary Artery Stenosis Estimates Made by Forensic Pathologists and Medical Students

*Mitchell Messner, BA\*, University of North Dakota SMHS, Grand Forks, ND 58202; Sarah Meyers, MD, Hennepin County Medical Examiner's Office, Minneapolis, MN 55415; Walter L. Kemp, MD, PhD, University of North Dakota School of Medicine, Grand Forks, ND 58202-9037*

**Learning Overview:** The goal of this presentation is to inform attendees about the lack of increased accuracy in estimating the percentage of stenosis that results from training and experience, in that forensic pathologists are not more accurate in this estimation than medical students.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how the accurate estimation of the percentage of stenosis produced by coronary artery atherosclerosis is an important step during the autopsy when determining the cause of death, with an appreciation that training and experience do not necessarily produce better estimates.

**Introduction:** An important step during the performance of an autopsy is the accurate estimation of coronary artery arteriosclerotic stenosis. Although tools, such as diagrams, exist to aid in improving accuracy, previous studies suggest that more experienced/specialized pathologists (i.e., cardiovascular pathologists) are less accurate than non-cardiovascular specialized pathologists (i.e., residents and clinical pathologists) in estimating the degree of coronary artery stenosis. To further explore these contradictory results, a study was designed to compare the accuracy of coronary artery stenosis estimates made by forensic pathologists versus medical students.

**Materials and Methods:** Six forensic pathologists and 12 medical students each independently examined 24 images of coronary artery cross-sections with variable degrees of stenosis and gave an estimate of the degree of arteriosclerotic stenosis. The medical students received no specific training prior to their review of the images. The images, including 12 gross and 12 microscopic images, were made available to each participant in a PowerPoint® presentation. The actual percentage of stenosis for each image was determined using ImageJ by measuring the area of the residual lumen and the area of the native lumen. To determine the area of the native lumen for calculation of the actual percentage of stenosis for the microscopic sections, both the outer edge of the intima and the outer edge of the media were measured. Given that the outer edge of the media was more apparent grossly, final analysis utilized the calculated value based on the outer edge of the media only. The median difference between the calculated percentage of stenosis using outer intima and outer media was 5.9%. The results were analyzed with R, with the non-parametric Wilcoxon Rank Sum Test used to determine statistical significance.

**Results:** Overall, when comparing all 24 images, the forensic pathologists had a median difference between the estimated and actual percentage of stenosis of -12.380 and the medical students had a median difference of -16.50 ( $p$ -value of 0.08542); however, for only the 12 gross images, the forensic pathologists median difference was -15.90 and the medical students was -20.90 ( $p$ -value of 0.2243), and for only the 12 microscopic images, the forensic pathologists median difference was -6.65 and the medical students was -13.050 ( $p$ -value of 0.08398).

**Conclusion:** In estimating the percentage of coronary artery arteriosclerotic stenosis, specific training in forensic pathology does not guarantee accuracy, with this study showing no statistically significant difference between estimates given by forensic pathologists or medical students and each group underestimating the degree of stenosis.

#### Coronary Artery, Stenosis, Atherosclerosis



### H129 Coronary Calcifications in Sudden Cardiac Death (SCD) Cases in Postmortem Computed Tomography (PMCT)

*Katarzyna Michaud, MD\**, Centre Universitaire Romand de, Lausanne 1000, SWITZERLAND; *Stefano Draisci, MD*, Dipartimento Interaziendale Ad Attività Integrata, Modena, ITALY; *Diego Proccichiani, MD*, Dipartimento di Diagnostica per Immagini, Modena 41100, ITALY; *Silke Grabherr, PhD*, Centre Universitaire Romand de Médecine Légale, Lausanne 25, Vaud 1000, SWITZERLAND; *Fabrice Dedouit, MD, PhD*, Centre Universitaire Romand De Médecine Légale, Lausanne 1000, SWITZERLAND

**Learning Overview:** After attending this presentation, attendees will understand modern postmortem imaging of coronary arteries. Attendees will understand how to evaluate the calcifications of coronary arteries of sudden death victims in PMCT and which cases could be a pitfall.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the advantages and possible pitfalls of modern radiological methods used in the evaluation of sudden death cases.

The Coronary Artery Calcium Score (CACS) is an independent predictor of coronary artery disease events in clinical practice and has been found to be a marker of a vascular injury that correlates closely with the atherosclerotic burden. Traditionally, coronary calcification is quantified radiologically by the Agatston score. The radiological grading of coronary artery disease, based on this score, is classified as no evidence of coronary artery disease (0 calcium score), minimal (1-10), mild (11-100), moderate (101-400), and severe (>400). In a living patient, a zero CACS is considered the most powerful negative risk factor for development of a coronary event and the assessment of CACS appears to be the most predictive in the intermediate-risk group. However, it is known that about 4% of patients present non-calcified plaques with a zero CACS.

In postmortem examination, it is possible to measure the CACS after the PMCT examination and before the opening of the body. However, up to the present, the CACS was not evaluated in postmortem practice. Therefore, the goal of this pilot study was to assess the CACS in PMCT in cases of SCD related to Ischemic Heart Disease (IHD).

Consecutive cases of SCD related to IHD for which the postmortem CT and CACS evaluations were available were retrospectively studied. The autopsies were performed in 2017 according to international guidelines. Violent deaths, such as hanging and gunshots, were included in the control group. The radiological examination was performed on a 64-row CT unit using a specific cardiac CT protocol with a non-enhanced sequential acquisition mode. The CACS was calculated by using the software Smartscore 4.0 from Advantage Windows® using a standard Agatston/Janowitz method. Board-certified radiologists trained in postmortem imaging assessed the CACS. All cases were classified into four groups according to the CACS as zero, 1-100, 101-400, and >400.

Twenty-five cases were selected in the study group; 5 women and 20 men; the mean age was  $63.2 \pm 13.3$  years. The CACS was 1-100 for 3 cases, 101-400 for 12 cases, and >400 for 10 cases. All female cases presented CACS below 400 and there were no cases with zero CACS. An acute coronary thrombosis was found in 14 cases, in 9 cases related to a rupture and in 5 cases to an erosion of a coronary plaque. In the control group, 27 cases were included (9 women and 18 men); the mean age was  $55.9 \pm 6.9$  years. The CACS was zero for 10 cases, 1-100 for 11 cases, 101-400 in 3 cases, and >400 in 3 cases.

This pilot study showed that the postmortem CACS was higher in the group of SCD related to IHD than in the control group. In 81.5% of the control population, the CACS was zero or below 100. The severe CACS (>400) was observed in IHD cases but also in controls. More interesting and representing the potential pitfalls, in 12% of SCD cases the CACS was mild, and in 48% of SCD cases it was between 101 and 400, corresponding to a moderate risk in clinical practice. This indicates that forensic pathologists and radiologists should evaluate very carefully all coronaries of sudden death victims, even if calcifications of coronary arteries are not very extensive in PMCT. The correlations found between postmortem imaging of SCD victims and autopsy could further the understanding of coronary syndromes in clinical practice.

#### Sudden Cardiac Death, Coronary Calcifications, Postmortem Imaging



### H130 Manual Large Volume DNA Extraction and Purification From a Large Substrate

Abigail Chesness\*, Oak Grove, MN 55303; Rachel L. Creager, Defense Forensic Science Center, Forest Park, GA; Thomas A. Meyer, MS, Atlanta, GA 30311

**Learning Overview:** After attending this presentation, attendees will understand a new method to extract and purify DNA from a small number of epithelial cells present on a large substrate.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new method for DNA extraction. Current extraction methods are not able to fully submerge large substrates in a single sample tube, and the use of multiple small sample tubes can result in DNA loss and/or contamination. The method in this study takes these issues into account to improve upon the effectiveness of extracting DNA from large substrates.

A new Manual Large Volume (MLV) DNA extraction method has been developed to efficiently and effectively extract and purify DNA from a large substrate. The standard automated extraction protocol for samples with low-level DNA (referred to as standard extraction protocol) doesn't work for large substrates because of the small sample tube size. Currently, the method used at the Defense Forensic Science Center for large substrates, referred to as the multi-tube method, divides a substrate into smaller pieces and extracts each piece individually using the standard extraction protocol. To compare different methods of DNA extraction and purification in this study, a stock solution of saliva diluted with TE buffer at a fixed ratio was used. By using this solution for each method, a percent recovery can be calculated based on the standard extraction protocol. The multi-tube extraction method results in 60%–70% DNA recovery compared to the standard extraction protocol, which can likely be attributed to increased sample manipulation.

The MLV method allows for the entire substrate to be submerged in lysis buffer in a single sample tube, reducing sample manipulation and dilution/concentration steps. The MLV method was compared to the standard extraction protocol and multi-tube method to determine its effectiveness in extracting DNA from large substrates. All methods utilize a lysis buffer with proteinase K and a binding buffer. The standard extraction protocol and the multi-tube protocol use a bead-based automated extraction, while the MLV protocol uses a silica column to separate and purify DNA.

The MLV extraction method resulted in 80%–90% recovery compared to the standard extraction protocol when a diluted saliva sample was used. The MLV extraction method was also shown to be effective for extracting from a paper substrate and was able to yield pure DNA samples at 80%–100% recovery relative to the standard extraction from paper. To give a better understanding of its overall effectiveness and reproducibility, the MLV extraction method will be tested using other substrates that mimic case work samples.

*The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army (DA) or the Department of Defense (DoD). Names of commercial manufacturers or products included are incidental only, and inclusion does not imply endorsement by the authors, the Defense Forensic Science Center (DFSC), the United States Army Criminal Investigation Command, the Office of the Provost Marshal General (OPMG), the DA, or the DoD.*

#### DNA Extraction, Large Volume, Manual Large Volume

### H131 Eye Temperature Measured After Death in Human Bodies as an Alternative Method of Time-of-Death Estimation in the Early Postmortem Period

Michał Kalisz, PhD\*, Gdansk 80-204, POLAND; Magdalena Wujtewicz, PhD, Medical University of Gdansk, Gdansk, Pomorskie 80-211, POLAND

**Learning Overview:** After attending this presentation, attendees will better understand the process of postmortem body cooling, particularly in the eyeball, and its application in the estimation of the Time Of Death (TOD), especially in the early, several-hours-long postmortem period.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the method of TOD estimation based on measurement of the postmortem eyeball temperature.

TOD estimation is a key matter for forensic pathologist examining the body at the death scene. In such cases, the body temperature should be measured aiming at TOD estimation. This is of great value when the body temperature is still higher than the environmental temperature and the TOD can be calculated on the basis of the difference between the body and the environmental temperature. However, despite the above and other possibilities of the TOD estimation (i.e., depending on postmortem changes such as lividity, rigor mortis or different supra-vital reactions, or even biochemical methods), they are still of not satisfactorily accurate.

In the current study, single (in 20 patients) or double (within a one-hour interval in 10 patients) eyeball and rectal temperature measurements were taken in patients who died in Medical University of Gdansk Hospital Intensive Care Unit. The actual TOD in each patient was exactly known and the body temperature was recorded shortly later (between 0.5h to 3.5h). The temperature was measured using pin probes connected to a high-precision electronic thermometer. The measured eye temperatures ranged from 29.7°C to 33.6°C. Ambient temperatures in all cases was stable (22°C), corresponding to usual room temperature. TOD was calculated using a formula based on Newton's law of cooling previously successfully applied in comprehensive studies on pigs and recent studies on human bodies:

$$t = - \frac{\ln\left(\frac{T - T_a}{T_0 - T_a}\right)}{k_c}$$

(equation 1)

where  $t$  is the time which passed since death (TOD),  $T$  is the temperature of the body site,  $T_a$  is the ambient temperature (constant: 22°C in the present study),  $T_0$  is the initial human eye temperature (assumed to be 35°C), and  $k_c$  is a first order cooling rate constant. Thanks to stable ambient temperature and knowledge of the exact TOD, the actual study allowed the adjustment of the mean value of  $k_c = -0.2 \text{ h}^{-1}$  in comparison to the recent studies. Thanks to both the significantly faster postmortem decrease of eye temperature and the residual or lack of plateau effect in the eye, and no influence of body mass, TOD in the human death cases using equation 1 could be estimated with quite a good accuracy. The maximum TOD estimation error during the postmortem intervals up to 3.5h was slightly higher than 1h minimum in 4 cases among 30, while for the rest of 26 cases it was less than 1h, while mean error for all 30 cases was  $\pm 24$ min. The results from 30 new cases with exactly known TOD show that the presented method of TOD estimation may be of satisfactory accuracy in the early postmortem period, particularly when applied to bodies found at room temperature and in standard environmental conditions.

#### Eye Temperature, Postmortem, Time-of-Death Estimation



### H132 Cold Case Homicides: DNA Testing of Retained Autopsy Sexual Assault Smears

*Michelle S. Clark, MS\*, Farmington, CT; Angela McGuire, MD, Office of the Chief Medical Examiner, Farmington, CT 06032; Kristin Sasinouski, MSFS, CT Division of Scientific Services, Meriden, CT 06451; James R. Gill, MD, Office of the Chief Medical Examiner, Farmington, CT 06032*

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**Learning Overview:** The goal of this presentation is to highlight the examination of retained medical examiner specimens for DNA testing and their use in cold case homicides.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the usefulness of testing archived autopsy sexual assault smears in identifying suspects in cold case homicides.

In 1994, the DNA Identification Act authorized the Federal Bureau of Investigation (FBI) to create a national DNA database of convicted offenders and databases for forensic samples. Prior to this, there was no consistent method for the collection, analysis, and comparison of biological specimens from suspected sexual assault-related homicides.

In the 1990s, vaginal, oral, and anal swabs and smears were collected on certain homicides at the Office of the Chief Medical Examiner (OCME) in Connecticut. The smear slides were reviewed by the medical examiner for sperm, then archived at the OCME. The swabs were sent to the crime lab for ABO blood type identification and possibly Polymerase Chain Reaction (PCR) testing. The crime lab began performing routine DNA testing on samples around 1993. Recent advances in molecular testing have allowed for the extraction of DNA to generate DNA profiles from cytological smears. Therefore, a search for archived vaginal, oral, and anal smear slides was undertaken at the OCME.

An electronic death certificate search was performed for all female homicides between 1990 and 1999. The OCME histology slide archives were searched for sexual assault smears for all 376 female homicides that occurred between 1990 and 1999. Of the 376 female homicides, the OCME had vaginal, anal, and oral slides on 84. Of these, 12 slides had sperm, 44 had no sperm, and 28 had no mention of the presence or absence of sperm in the report. Detectives from the jurisdictions of death were contacted to see if any of the cases were still unsolved. Of the 12 instances with sperm, there were 7 that were still “unsolved.” In these instances, samples were forwarded to the Connecticut State Division of Scientific Services (DSS) for DNA testing and analysis by extraction of DNA from cells on the previously stained and fixed glass slides.

DNA profiles were obtained on all seven cases. Two profiles entered into the Combined DNA Index System (CODIS) resulted in two matches (“hits”). One profile was consistent with the decedent’s husband (who was the suspect). This information was provided to the investigating police agency. Two profiles were mixtures that were not entered into CODIS (vaginal smear with three male contributors and vaginal and oral smear with mixtures of two individuals (one male)). In one case, vaginal swabs detected three contributors (two were male), and the vaginal smear had two contributors (one male). CODIS entries were made for both without any hits. In one case, the vaginal swabs, which included a swabbing of the vaginal smear, detected three contributors (one male), and a CODIS entry was made without any hits.

This review of archival sexual assault smears resulted in DNA profiles that were able to assist in the investigation of three cold case homicide investigations in Connecticut. Given the current advances in both DNA extraction techniques and molecular analysis, medical examiner offices may wish to search their archival histology slides for sexual assault smears that may yield sufficient sperm for DNA analysis on previously unsolved homicide cases.

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#### DNA, Sexual Assault, Autopsy



### H133 Normalization of Polymerase Chain Reaction (PCR) -Based Quantification Using 9947A Human Standard DNA

Grace S. Woods, BS\*, Pleasant Garden, NC 27313; Rachel L. Creager, Defense Forensic Science Center, Forest Park, GA

**Learning Overview:** After attending this presentation, attendees will better understand the use of 9947A human standard DNA as a normalization control for comparing data from multiple quantitative PCR (qPCR) plates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by creating a method that increases the ability to compare DNA concentrations collected via qPCR on different days or when standards are used toward the end of the recommended storage life.

This study evaluated the effectiveness of using purchased 9947A human standard DNA as a normalization control for Plexor® HY and Quantifiler® Trio quantification kits. Currently, there is no normalization control included in qPCR kits for plate-to-plate comparisons when data is collected on different days or across multiple plates. Differences in quant values when comparing qPCR data collected in different runs may arise for a range of reasons, including differences in the dilution accuracy of the standards, pipetting accuracy of the amplification mix, or because the standard dilutions are near the protocol-specified storage limit and may be degraded. To assess some of these variations, a comparative analysis of quantification standards over time was performed using purified 9947A human standard DNA as a normalization control.

Quantification standards from Plexor® HY and Quantifiler® Trio kits were made and assessed at time points up to 14 days. Each set of standards was plated with purified DNA samples and 9947A human standard DNA using the QIAgility® automated robot. The observed quant value for 9947A can then be used in relation to its known concentration to normalize the sample concentration and allow for a more accurate comparison of quant values. Preliminary data shows that aged standards artificially increase the DNA concentration of plated samples. However, when using 9947A as a normalization control, data can be corrected to real concentration values. The effect of this normalization strategy will be discussed for samples across a wide range of quant values.

Based on the trends of qPCR standards degrading as they approach the recommended storage life and the inherent variables present in the quantification process, a normalization technique is necessary to compare data across multiple plates or from multiple days. This research has shown that artificially increased or skewed DNA concentrations can be corrected to their true value by using purified human standard DNA with a known concentration as a normalization control. Results from this study show this normalization method is useful for longitudinal research studies looking at the effect of modifications to sampling, storage, or extraction procedures.

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#### DNA Quantification, Normalization, qPCR





### H134 Body Coloration Artifacts in Forensic Autopsy

Asit Kumar Sikary, MD\*, ESIC Medical College & Hospital, Faridabad, Haryana 121001, INDIA; Chittaranjan Behera, MD, Forensic Medicine, All India Institute of Medical Science, New Delhi, Delhi 110029, INDIA

**Learning Overview:** After attending this presentation, attendees will understand the various body color artifacts that are introduced to the body before or after death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enabling attendees to differentiate color artifacts from genuine antemortem or postmortem color changes occurring in the body.

Skin and mucosal discoloration are common in various diseases and poisonings. Jaundice, hemochromatosis, cyanosis, polycythemia, pancreatitis, retroperitoneal hemorrhage, carbon monoxide, and cyanide poisoning, etc. impart characteristic discoloration to the skin and mucosa, directing the autopsy surgeon for particular pathology, injury, or poison. It is thus highly important to be aware of coloration artifacts introduced to the external body surface leading to the wrong interpretation of various antemortem and postmortem findings.

Cases of the past 12 years from South Delhi, India, in which color was applied over the body surface before or after death formed the study group. The data were retrospectively collected from the archive of the center, including postmortem reports, inquest papers, and photographs of the cases.

One of the major scenarios of skin coloration in the present study was Holi, a color festivity of Hindus. These colors, when freshly applied, can be differentiated from skin changes; however, attempts to wash the body leaves a tinge of the color and may cause misinterpretation of deep facial congestion with red colors; of early bodily decomposition with green colors; of postmortem lividity over limbs or trunk with purple colors; and of cyanotic changes with blue colors. However, differences can be made by further fading of the color with washing, unusual appearance, or site of the color, and having no correlation with the bodily appearance, condition, injury, or disease.

Another major source of dead body coloration was the after-death ritual of applying turmeric paste. Its color is faded away when scrubbed thoroughly with hot water, leaving a yellow tinge, and may cause misinterpretation with signs of jaundice but can be distinguished by being unusual in site and distribution, no icterus, and no medical or surgical pathology known to cause jaundice.

Beauty products used by the deceased, such as vermilion and black eyeliner, was another source of discoloration. These beauty products always leave traces when cleaned or washed. Traces of eyeliner around eyes lead to misinterpretation, such as a black eye seen in head injury. However, proper history about the application of the product, regarding head injury and anatomical dissection, rules out the black eye phenomenon. Traces of smudged vermilion over the forehead causes confusion with facial congestion. However, it has an unusual distribution and fades away when washed.

Worn clothing stained the body surface as the clothing color was not fast enough. Likewise, ligature material used for hanging stained the ligature mark. These clothing-related colorations of the body or ligature mark are helpful in determining worn clothes or ligature material in doubtful cases. These colors are usually faded in appearance and distribution of the color is over the body regions covered by the cloth. Their colors are washed away with thorough hot water scrubbing. However, being faded in appearance, no one suspects it as an external coloration and, if not thoroughly washed, leads to various misinterpretations, depending on the color.

Finger and palm printing with blue pad ink was another source of body coloration, which is a standard procedure for identification. The ink leaves traces even after thorough hot water scrubbing. The discoloration of the palm can cause a misinterpretation of cyanosis, more so when fingernails are mistakenly stained during the procedure. However, other sites will not show any cyanotic change.

The medicinal dyes povidone iodine and methylene blue have discolored parts of the limbs. These were used for a wound which stained the skin, reddish-brown and greenish blue, respectively. These stains fade with hot water wash and can be misinterpreted as contusion and gangrenous lesions, but regional dissection at autopsy will clear the picture.

If the autopsy surgeon sees the body before washing of the color or takes the history about external coloration whenever there is unusual body coloration, the misinterpretation can be avoided. Touching these types of external coloration, if the color is not completely dried, with a gloved hand or a cotton swab will automatically stain the glove or swab. Examination of clothing will show the discoloration too.

#### Postmortem Change, Postmortem Artifact, Misinterpretation



### **H135 A Study on Detection of A-Helix Protein in Post-Traumatic Epileptogenic Focus by Fourier Transform Infrared (FTIR) Mapping**

*Bin B. Dai\**, Beijing, AB, CHINA; *Siyang Xiang*, Beijing, ASIA, CHINA; *Xu Wang, MD*, China University of Political Science and Law, Beijing, CHINA; *Tiantong Yang*, Haidian District, Beijing 100192, CHINA

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**THIS ABSTRACT WAS NOT PRESENTED.**



### H136 Trends in Novel Opioid Use in the United States From January 2014 Through April 2018 in Medicolegal Death Investigations (MDIs) and Strategies to Improve Early Identification, Detection, and Interpretation

*Barry K. Logan, PhD\*, NMS Labs/Center for Forensic Science Research and Education, Willow Grove, PA 19090; Sherri L. Kacinko, PhD, Willow Grove, PA 19090; Donna M. Papsun, MS, NMS Labs, Willow Grove, PA 19030; Amanda L.A. Mohr, MSFS, Center for Forensic Science Research and Education, Willow Grove, PA 19090*

**Learning Overview:** After attending this presentation, attendees will be able to describe the trends in novel opioids involved in MDI cases between 2014 and 2018, and the structural modifications made to the fentanyl molecule and its implications for toxicity. Attendees will be able to verify that the testing protocols being used in MDI investigations are appropriate and fit for purpose.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating death investigation professionals, coroners, and medical examiners about how to ensure that appropriate toxicology is being performed on their casework and that current and emergent drugs will be detected to assist with the appropriate certification of death and the collection of accurate death statistics.

The current opioid epidemic had its roots in the overprescribing of prescription semi-synthetic opioids in the 1990s and 2000s. Subsequent reductions in the legal availability of these drugs contributed to a demand for illicit opioids, including more easily imported illicit fentanyl. Once the infrastructure was in place to manufacture illicit fentanyl, it was an easy step for the clandestine manufacturers to make different variants with both greater and lesser potency. The same infrastructure allowed for the manufacture of completely novel opioids from the pharma back catalog also.

The impact of this has created a significant challenge for toxicology laboratories in creating and maintaining a relevant scope of analysis, including challenges of what to test for, the appropriate technology to use, the regulatory/accreditation environment, new drug trend intelligence, and availability of standard reference materials.

The early trends in novel opioids included the appearance of acetylfentanyl in 2014, followed by U47700 and furanylfentanyl in 2015, carfentanil in late 2015 and through 2016, then a plethora of boutique fentanyl analogs in 2016 and 2017, some of which persist at lower frequency today. Laboratory trend analysis indicates that while there is no end in sight for opioid abuse-related deaths, in the first half of 2018, it appears that death rates of cases involving opioid abuse have plateaued.

This presentation will review the relative frequency of novel opioid detection in death investigation casework and the concentrations and estimates of the toxicity of the various analogs detected. This presentation will discuss the meaning of cases with the presence of apparent fentanyl metabolite (4-ANPP) but no fentanyl and the need to perform further testing in these cases. This presentation will review the current trends toward toxicities associated with combinations of drug classes, including opioids and benzodiazepines, opioids and stimulants, and opioids and synthetic cannabinoids.

The application of high-resolution time-of-flight mass spectrometry has enabled toxicology laboratories to look retrospectively at the presence of newly discovered drugs in older casework without having to retest the samples and the use of data archiving as a tool for understanding the pharmacoepidemiology of emerging drug toxicity outbreaks.

This presentation will conclude with the description of a model of integrating data from seized drug investigations, MDI casework, and investigations of drug toxicity outbreaks in emergency room populations to support a system for early identification of new substances, notification of stakeholders in public health and public safety, and an early warning system to alert death investigators and toxicology laboratories to emerging trends.

#### Drug Early-Warning System, Opioids, Drug-Related Death



## H137 Acetaminophen Toxicity Deaths in New Mexico: 1990–2016

Lori A. Proe, DO\*, Office of the Medical Investigator, Albuquerque, NM 87102; Sarah Lathrop, DVM, PhD, Albuquerque, NM 87111

**Learning Overview:** After attending this presentation, attendees will understand the nature and scope of acetaminophen toxicity in the United States, interventions to reduce it, and specifics about trends of acetaminophen toxicity deaths in New Mexico.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) providing information on the significance of acetaminophen overdoses in the United States, (2) explaining efforts to reduce acetaminophen toxicity, and (3) discussing trends in acetaminophen toxicity deaths in one statewide medical examiner's office.

**Background:** Acetaminophen is the most commonly used pain reliever in the United States and is found in more than 600 medications.<sup>1,2</sup> At high doses, acetaminophen is metabolized to N-Acetyl-P-benzoquinoneimine (NAPQI), a hepatotoxin.<sup>1</sup> According to the Acute Liver Failure Study Group, most of the approximately 2,000 yearly cases of acute liver failure are caused by acetaminophen toxicity.<sup>3</sup> At least as early as 1977, the United States Food and Drug Administration (FDA) recognized the potential danger of acetaminophen and recommended that warning labels be added to acetaminophen-containing products.<sup>4</sup> Beginning in 1998 and as recently as 2011, the FDA issued rules and recommendations for labeling and dosing of prescription and over-the-counter drugs with warnings about the dangers of acetaminophen hepatotoxicity.<sup>5-7</sup> However, fatalities involving acetaminophen continue and may have increased between 2000 and 2009.<sup>8</sup>

**Purpose:** The goal of this project is to study deaths occurring as the result of acetaminophen toxicity in New Mexico to look for overall trends and to determine whether warning label interventions from the FDA affected the numbers of people dying from acetaminophen toxicity.

**Methods:** This study queried the New Mexico Office of the Medical Investigator's (NMOMI) electronic database for all deaths having acetaminophen in the Cause of Death section from 1990 through 2016. Cases were reviewed for relevance and cleaned in Excel®. Analysis was performed using Statistical Analysis Software (SAS) version 9.4. Categorical variables were compared using either a chi-square test or a Fisher exact test if an expected cell count was less than 5. Continuous variables were compared using a Wilcoxon rank-sum test, or a Kruskal-Wallis for multiple comparisons. P-values of 0.05 or less were considered statistically significant.

**Results:** Between 1990 and 2016, there were 158 acetaminophen-related NMOMI deaths, with a peak number of cases (18) in 2000. In most cases (98, 62%), acetaminophen was ingested with other substances. Hepatic toxicity was significantly more likely if acetaminophen was ingested alone and if alcohol use contributed to death ( $p < 0.0001$  for both). Declines in acetaminophen toxicity deaths were not observed in the years following labeling changes for acetaminophen-containing products. American Indians died from acetaminophen toxicity at significantly younger ages than those in other racial/ethnic groups ( $p < 0.0001$ ).

**Conclusions:** There appears to be no connection between labeling changes for acetaminophen-containing products and the number of acetaminophen toxicity deaths.

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### Acetaminophen, New Mexico, Toxicity



### H138 Comparative and Correlation Studies of Biochemical Substances in Vitreous Humor (VH) and Synovial Fluid (SF)

*Supawon Srettabunjong, MD\**, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, THAILAND; *Wantawanop Thongphap, BSc\**, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkoknoi 10700, THAILAND; *Anchalee Chittamma\**, Faculty of Medicine Ramathibodi Hospital, Mahidol, Ratchathevi 10400, THAILAND; *Wanna Thongnoppakhum\**, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkoknoi 10700, THAILAND

**Learning Overview:** After attending this presentation, attendees will better understand the comparative study of certain biochemical substance levels between two eyes and two knees of the same subject, and between the two compartments, as well as the correlation study between such biochemical substance concentration in VH and SF of the same person, and the essential basic knowledge for forensic application and research when using biochemical substance level in either fluid as a substitute body fluid for unavailable or inadequate blood to solve any forensic issues in daily practice or further research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting critical information of biochemical substance investigation in VH and SF for forensic purposes as it can augment traditional means of forensic investigation.

In forensics, in the case of a lack of blood, the VH and SF become optional postmortem samples. However, few studies have explored SF as a potential source for postmortem biochemistry investigation, and there is still controversy around whether the biochemical concentrations in the VH in both eye compartments are the same. In this study, the concentrations of certain biochemical substances in both VH and SF were evaluated and determined to compare their concentrations: (1) between the VH and SF from both sides, and (2) between the VH and SF themselves. The concentrations of the biochemical substances in both fluids were also evaluated for their correlation. VH and SF samples were collected from 35 cadavers (28 males and 7 females) with the mean age of  $47.3 \pm 16.5$  years (range 18–88 years); all subjects had died within 8h before their samples were collected. The samples were centrifuged, and the supernatant fluids were used for biochemical analysis.

The results indicated no statistically significant difference in the concentrations of all the biochemical substances studied between the VH and SF from both sides ( $p > 0.05$ ). Sodium, potassium, chloride, and magnesium concentrations were significantly higher in the VH than those in the SF, whereas uric acid and creatinine concentrations were higher in the SF compared with the VH. The concentrations of sodium, potassium, glucose, lactate, urea, uric acid, and creatinine between the VH and SF showed statistically significant correlation ( $p < 0.005$ ), but not chloride and magnesium concentrations. The results showed that VH or SF samples from either side can be used for postmortem biochemistry analysis. In addition, due to the significant relationships of the changing concentrations of all biochemical substances studied (except chloride and magnesium) between the VH and SF, the SF can be used as an optional postmortem sample for certain biochemical measurements in forensic applications and further research.

**Postmortem Biochemistry, Postmortem Chemistry, Thanatochemistry**



### H139 The Outbreak of Fentanyl-Related Deaths in Cook County, Illinois, Between October 2015 and December 2017: A Retrospective Study and a Comparison With Previous Data

Lorenzo Gitto, MD\*, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210; Steven M. White, MD, PhD, Cook County Medical Examiner's Office, Chicago, IL 60612; Ponni Arunkumar, MD, Cook County Medical Examiner's Office, Chicago, IL 60612; Serenella Serinelli, MD\*, State University of New York Upstate, Department of Pathology, Syracuse, NY 13210

**Learning Overview:** After attending this presentation, attendees will better understand the prevalence of fentanyl-related deaths in Cook County, IL, and what drugs are being used with fentanyl.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the recent increase of fatalities due to fentanyl intoxication compared to previous data obtained in the same area.

Fentanyl is a synthetic  $\mu$ -agonist, a phenylpiperidine derivative with a structure closely related to that of meperidine. It is a potent narcotic used for anesthesia and analgesia. Individuals who are prescribed fentanyl for pain are prone to abuse or become addicted to the drug.

Moreover, fentanyl is made illegally and used as a recreational drug, often mixed with heroin or cocaine, since low production costs encourage suppliers to "cut" recreational drugs with it.

Previous research in Cook County found that a cluster of deaths from fentanyl intoxication appeared in late 2005. The study was conducted by Denton et al. between September 2005 and April 2007 and showed approximately 350 fentanyl-related deaths occurred in Cook County.<sup>1</sup> This epidemic had a peak in the middle of 2006, then drastically terminated in early 2007. In recent years, a new epidemic of fentanyl-related fatalities has been observed in Cook County.

To estimate the number and features of deaths due to fentanyl intoxication during the new epidemic, the electronic database of the Cook County Medical Examiner's Office in Chicago was examined for cases of people dying from fentanyl toxicity or combined drug toxicity, including fentanyl, between October 2015 and December 2017. A research using the keyword "fentanil" was also performed in the same period. The data were reviewed for age, sex, race, and cause and manner of death. The fentanyl-related deaths were then subcategorized as to the presence or absence of other drugs. No limits were imposed.

A total of 27,131 cases were identified in the studied period. Among these, 1,256 cases contained the keyword "fentanyl" and 4 cases contained the keyword "fentanil" as a primary cause of death. This study detected 112 cases of fentanyl deaths without other drugs or substances, 147 cases of fentanyl + heroin, 54 cases of fentanyl + ethanol, and 37 cases of fentanyl + cocaine. The remaining 900 cases included multiple drug intoxications (more than two drugs) with fentanyl synthetic analogues and other less frequent drug combinations. In four cases, the death was due to a combination of drugs including carfentanil but no fentanyl. A thorough discussion of the results, including demographic data and the comparison with the previous epidemic, will be shown to attendees.

After a first epidemic between 2005 and 2007, fentanyl intoxications significantly reduced, then reappeared in the recent years. Knowledge about this new outbreak could be useful for public health in monitoring and quickly diagnosing and treating acute intoxication when fentanyl is involved. Moreover, knowing the extent of the current problem could be useful for public safety purposes, while the analysis of the recreational drugs usually combined with fentanyl can contribute to a better-informed public policy that helps reduce risk for intravenous drug abusers.

#### Reference(s):

1. Denton J.S., Donoghue E.R., McReynolds J., Kalelkar M.B. (2008). An Epidemic of Illicit Fentanyl Deaths in Cook County, Illinois: September 2005 Through April 2007. *J Forensic Sci* 53:452–4541.

#### Fentanyl, Toxicity, Synthetic Opioids



## H140 Loperamide Intoxication: Case Reports and a Review of the Literature

Paul Uribe, MD\*, Martin Army Community Hospital, Fort Benning, GA 31905

**Learning Overview:** After attending this presentation, attendees will (1) better understand the pharmacology and physiology of loperamide toxicity, (2) know the incidence of use and misuse of this drug for managing opiate withdrawal symptoms, and (3) be aware of the implications of the 2016 adverse side effect ruling on loperamide by the Food and Drug Administration (FDA).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the growing problem of toxicity and lethality of this readily available over-the-counter medication.

Loperamide is a readily available and increasingly abused over-the-counter anti-diarrheal medication that is a growing cause of medication-related fatalities in the United States. In higher doses, loperamide can produce side effects of cardiotoxicity, cardiac arrhythmias, euphoria, and central nervous system depression. Loperamide is a  $\mu$ -opioid receptor agonist that is metabolized by the cytochrome P450 system.<sup>1</sup> Loperamide has low bioavailability in appropriate doses, and therefore has limited central nervous system activity due to the limited ability to cross the blood-brain barrier. In higher doses, the drug can penetrate the central nervous system and lead to tolerance and, upon cessation of use, withdrawal symptoms. The arrhythmogenic side effects include syncope, QT-interval prolongation, QRS-interval widening, torsade de pointes, ventricular dysrhythmias, and cardiac arrest.<sup>2</sup> Loperamide is increasingly used, and misused, as an inexpensive, readily available, over-the-counter alternative for self-managing the symptoms of opiate withdrawal, including withdrawal from methadone.<sup>2-4</sup>

From 2010 to 2015, there was a 91% increase in reported loperamide abuse.<sup>5</sup> Between 2002 and 2015, 224 adults were referred to a health care facility for intentional ingestions of loperamide as reported to the poison control system.<sup>6</sup> During this period, there were 15 reported deaths involving loperamide, with over half involving single-agent loperamide abuse. There was a spike in the number of cases between 2014 and 2015, which coincided with plentiful online instructions on how to abuse this drug. The availability and misuse of loperamide has resulted in the FDA issuing a warning about the adverse side effects in June 2016.<sup>7</sup> This warning was based on 48 cases of severe cardiac side-effects associated from loperamide misuse from the approval date of loperamide of December 1976 through December 2015, with more than half of the cases reported after 2010.

Depending on what toxicology screens are available, loperamide may be overlooked as routine drug screen panels may not detect this drug. It may also be clinically overlooked in routine drug testing for drug-treatment program compliance. Thorough scene investigation may overlook this relatively innocuous medication. Clinical awareness of this entity is critical to early recognition and treatment. Two unexpected deaths from acute loperamide intoxication are discussed and followed by a discussion of the pharmacology, side-effects, and abuse trends involving this readily available over-the-counter medication.

### Reference(s):

1. Bishop-Freeman S.C., Feaster M.S., Beal J., Miller A., Hargrove R.L., Brower J.O., and Winecker R.E. Loperamide-Related Deaths in North Carolina. *Journal of Analytical Toxicology*, 2016; 40: 677-686.
2. Wu, P.E., Juurlink D.N. Clinical Review: Loperamide Toxicity. *Ann Emerg Med*, 2017 Aug; 70(2): 245-252.
3. Vithalani N.D., Heron C., Rao R.E., Cardell A.F., Stephens M.B. Dysrhythmias With Loperamide Used for Opioid Withdrawal. *J Am Board Fam Med*, 2017 Nov-Dec; 30(6): 832-834.
4. Miller H., Panahi L., Tapia D., Tran A., Bowman J.D. Loperamide Misuse and Abuse. *J Am Pharm Assoc* (2003), 2017 Mar-Apr; 57(2S): S45-S50.
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6. Eggleston W., Marraffa J.M., Stork C.M., Mercurio-Zappala M., Su M.K., Wightman R.S., Cummings K.R., Schier J.G. Notes From the Field: Cardiac Dysrhythmias After Loperamide Abuse—New York, 2008-2016. *MMWR Morb Mortal Wkly Rep*, 2016 Nov 18; 65(45): 1276-1277.
7. Eggleston W., Clark K.H., and Marraffa J.M. Loperamide Abuse Associated With Cardiac Dysrhythmia and Death. *Ann Emerg Med*, 2017 Jan; 69(1): 83-86.

### Loperamide, Toxicology, Cardiotoxicity



### H141 Cocaine as the Sole Intoxicant in an Unusual Suicidal Overdose

*Michael Heninger, MD\*, Fulton County Medical Examiner's Office, Atlanta, GA 30312*

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**Learning Overview:** After attending this presentation, attendees will understand that cocaine can be the sole intoxicant in a suicidal overdose.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing how cocaine can be the sole intoxicant in a suicidal overdose in the circumstances of an unusual personal belief system.

Drug overdoses have become far more common in the United States in the past few years. Recreational drugs have previously often been used repeatedly in accidental overdoses with the intent of no serious lasting consequences. Drugs selected in suicide are thought to be dangerous by the users who desire the most serious consequence. Although there is considerable overlap between the drugs commonly used in suicidal versus accidental overdoses, the use of perceived less-dangerous stimulants is rare in suicides.

Cocaine is often the only drug detected in an accidental overdose and frequently one of many in either a suicidal or accidental overdose. Cocaine can be inhaled, snorted, injected intravenously, ingested orally, or absorbed vaginally or rectally, all with potentially fatal outcomes. Cocaine as the only potentially lethal substance used to commit suicide is rare. No other cases are known in per this research in a setting with thousands of accidental cocaine deaths over many decades.

Reported is an intentional overdose using cocaine as the only dangerous substance of a 37-year-old Black female, who was otherwise in excellent health. The decedent was found by her mother, expired in her bed in a prone position with her face in a pillow. Paramedics were summoned and noted that she was in rigor and did not attempt to resuscitate her. Pink fluid stains were on the pillow under her face. A container with only a small amount of residual pink fluid made cloudy with a white residue was found. Drug paraphernalia, including a small clear plastic bag with small white rocks and trace amounts of residual white power along with green leafy vegetable material, was noted. Also obtained was a small container of commercially available of 24K edible gold leaves. The decedent had a history of schizophrenia and/or bipolar disorder with previous suicidal ideation.

The lab results showed a cocaine level of 2,500ng/ml, benzoylecgonine level of 4,100ng/ml, and a cannabinol level of 1.5ng/ml.

The intent was well documented with a mysterious handwritten note of several pages entitled "Death Ritual Protocol." Select quotes included: "Invocation of Eji Ogbe accompany me on the road," "Eat drugs save cocaine for last snort as much as possible," and "My father will come to life with me. The ground will open up." Web-based searches of several of the proper names listed in the note indicated they are part of a religion or system of divination called Ifa. It appears to have originated in West Africa among the Niger-Congo ethnic group centuries ago and is practiced there and throughout the Americas. It plays a role in many syncretic religions such as Santeria, Candomble, Palo, Umbanda Vodou, and others. The notes indicated a clear intent to die, but also the intent to not stay dead.

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### Suicide, Cocaine, Overdose





### H142 The Utilization of Preliminary Urine Drug Screens in Heroin- and Fentanyl-Related Deaths

*Gerald Feigin, MD\*, Sewell, NJ 08080*

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**Learning Overview:** The goal of this presentation is to show how to use preliminary urine drug screens in routine drug-related deaths to help rapidly and accurately determine a reasonable initial cause of death.

**Impact on the Forensic Science Community:** Pending every drug-related death causes undue difficulties for families of decedents. This presentation will impact the forensic science community by demonstrating how to use preliminary drug screens to create a cause of death on a death certificate. Follow-up and amendments can be made after final toxicology is received.

Positive urine drug screens rapidly and accurately predict findings in postmortem confirmatory toxicology and avoid pending cases.

An examination of 200 consecutive heroin- and fentanyl-related deaths shows a very strong correlation between preliminary urine screen for heroin (opiates). Fentanyl, because of the very rapid respiratory depression, often may not be present in urine screens. However, using a “cheat” with minimally diluted cardiac blood often shows fentanyl positivity. When drug-related deaths have negative results on preliminary screens, novel fentanyl analogs are often present and frequently require more extensive toxicologic testing. Although a urine drug screen is not a substitute for postmortem blood toxicology, it is a very good tool for rapid guidance in determining cause of death.

Cocaine, methamphetamine, methadone, and benzodiazepines, such as alprazolam, clonazepam, and diazepam, were not considered for this presentation. They clearly contribute to cause of death either directly or indirectly. The screen that was used will detect cocaine metabolite, methamphetamine, opiates, and benzodiazepines. A separate screen is used for fentanyl. Cocaine is often seen in combination with all of the above. A separate presentation will be made in regard to combination drug deaths in the future. It should be noted that there is no therapeutic level of heroin, cocaine, or fentanyl when used in non-medical circumstances.

When death certificates are pending, this causes stress to families who have already lost a loved one. Waiting for final toxicology to be received can take weeks to months depending upon jurisdiction. This prevents families from receiving death benefits, funeral directors from being paid, and children of decedents from receiving needed money. Using preliminary drug screens, when positive, to create an initial cause of death such as “Adverse Effect of Drugs” on a death certificate, followed up by a more precise cause of death by listing all the responsible agents is a reasonable alternative to universally “pending” every and all drug deaths at time of autopsy. This also simplifies amendments by allowing only one line to be changed, the cause line, as opposed to all other items in amendment such as how, where, when did injury occur.

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#### **Preliminary Drug Screen, Heroin, Fentanyl**



**H143 WITHDRAWN**



### H144 A Modern Application of Forensic Anthropology in Coroner and Medical Examiner Offices: A Preview

*Katharine Chapman Pope, MA\*, Division of Forensic Science, Wilmington, DE 19801; Lindsay H. Trammell, PhD, Saint Louis County Office of the Medical Examiner, St. Louis, MO 63134; Sharon M. Derrick, PhD, Texas A&M University-Corpus Christi, Corpus Christi, TX 78412; Elizabeth A. Miller, PhD, California State University Los Angeles, Los Angeles, CA 90032*

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**Learning Overview:** The goals of this presentation are to: (1) inform the medicolegal community of the value of forensic anthropology in death investigation, (2) apprise attendees of the details of obtaining valuable assistance in casework from a forensic anthropologist, (3) discuss the certification process within the field, and (4) impart through case examples exactly how forensic anthropologists can assist them in their casework.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the utility of forensic anthropologists in daily tasks of a medical examiner/coroner office.

Forensic anthropologists are gaining attention in the medicolegal community as an underutilized resource whose multi-layered approach to death investigation can be invaluable. Medicolegal death investigators, law enforcement, medical examiners/coroners, criminalists, attorneys, and judges often rely on discipline-specific experts to enhance their expertise. Forensically trained anthropologists are knowledgeable in all aspects of death investigation and can assist in field recovery of decedents, classification of cause and manner of death, identification of unknown decedents, and prosecution or defense of suspects in court. Although anthropologists began serving as consultants in death investigations much earlier, forensic anthropology as a discipline was recognized by the American Academy of Forensic Sciences (AAFS) in the mid-1970s, and the American Board of Forensic Anthropology (ABFA) came into existence in 1977.

Forensic anthropologists are experts in locating clandestine graves and recovering remains from a variety of contexts, such as fire scenes, graves, surface scatters, collapsed buildings, plane crashes, or any scene in which a body is not recently deceased, fully visible, and/or intact. With their knowledge of osseous/cartilaginous tissue anatomy and archaeological recovery methods, anthropologists can differentiate non-human remains during scene searches, and recover remains and associated evidence under circumstances far removed from the routine death scene. These skills save time and effort for each agency involved.

From the autopsy table to the laboratory, the forensic anthropologist can provide a full spectrum of analyses, from biomechanical evaluation of fractures to timing of injuries and healing, to differential diagnoses of pathological conditions. At the request of the medical examiner/coroner, the anthropologist may retain specimens from a fleshed body and remove soft tissue for analysis of the bone or cartilage. Anthropological assessments may establish patterns of trauma in child or elder abuse cases or differentiate inflicted from accidental injury. In skeletal cases, the anthropologist can sort commingled remains into individuals, estimate the number of individuals represented, construct demographic profiles, and reconstruct what happened to the remains from the time of death to the time of recovery, thereby enhancing the opportunity for personal identification. Following analysis, the anthropologist can properly curate skeletal remains and reinvestigate unidentified cold cases.

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### Medical Examiner/Coroner Office, Forensic Pathology, Forensic Anthropology



### H145 The Histomorphology of Cranial Fracture Healing: Case Examples

*Carolyn V. Isaac, PhD\*, Kalamazoo, MI 49008-8074; Jered B. Cornelison, PhD, Western Michigan University School of Medicine, Kalamazoo, MI 49008; Joseph A. Prahlow, MD, Western Michigan University School of Medicine, Kalamazoo, MI 49007*

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**Learning Overview:** After attending this presentation, attendees will understand the types of histologic changes that are manifest within healing cranial fractures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the histologic features of several examples of healing/healed cranial fractures, including samples from differing age groups, different fracture/healing time, and varying types of histology stains used.

This study recently received a National Institute of Justice grant for the investigation of cellular and morphologic characteristics of healing cranial vault fractures, to develop a time-since-fracture database specifically related to cranial fractures. This database is composed of cranial fractures with a known date and time of injury, with samples submitted to the Western Michigan University School of Medicine from numerous partner offices/institutions. The development of specific protocols for radiologic evaluation, as well as sample decalcification and processing are ongoing. A series of several stains, including hematoxylin and eosin, trichrome, and Alcian Blue/Orange G, are prepared for each sample, and a group of blinded forensic pathologist and anatomist reviewers evaluate the histologic sections for the presence or absence of numerous specific features. As the total number of samples submitted increases, the hypothesis is that a reliable, reproducible, histomorphological timeline for cranial fracture healing will be established, with possible notable variations related to fracture location, concomitant morbidities, and age of patient/decendent. This time-since-fracture database will be helpful in evaluating other forensic cases, where the timing of cranial fracture is in question.

As arrangements with partner organizations are ongoing and sample preparation protocols are being refined, the database currently contains approximately two dozen samples, with decedent ages ranging from approximately 2 months to nearly 78 years of age and known injury ages ranging from acute (minutes) to 42 years. In this initial report, this study will present examples of the types of cases currently in the database. These will include cases of acute fractures, where virtually no histologic evidence of healing exists, cases with several hours of healing, cases with days-to-weeks-to-months of healing, and cases which are, for all practical purposes, considered completely healed, with a post-injury healing time of many years. Photomicrographs of histology sections with each stain will be provided, focusing on the important aspects of the healing process that are highlighted by each stain.

Although one aim of this presentation is to provide an illustration of the histologic features of cranial fractures of varying age and in different aged cohorts, an additional goal is to use the presentation as an outreach to the forensic pathology community to recruit more partner offices/institutions for this important project. Only with a sizeable sample will this project attain the numbers necessary for reliable statistical conclusions. Cases of healing cranial fractures of definite known time-of-injury are not very common; however, with more partners involved in the study, the hope is that an adequate sample size will be attained so that this time-since-fracture database will represent a solid and reliable resource for forensic scientists faced with questions regarding cranial vault fracture healing time.

*This research project is supported by funding provided by a National Institute of Justice grant.*

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### **Cranial Vault, Fracture, Time-Since-Injury**



### H146 A Comparison of Three Methods for Assessment of Bone Decalcification Time and Quality of Histological Slides for Cranial Fracture Healing Investigation

Jered B. Cornelison, PhD\*, Western Michigan University School of Medicine, Kalamazoo, MI 49008; Carolyn V. Isaac, PhD, Kalamazoo, MI 49008-8074; Joseph A. Prahlow, MD, Western Michigan University School of Medicine, Kalamazoo, MI 49007

**Learning Overview:** After attending this presentation, attendees will: (1) learn an easy, accurate, and non-destructive method for determining whether bone samples are decalcified; (2) learn the amount of time required to decalcify cranial vault bone using three different decalcification methods among three different age cohorts; and (3) understand which decalcification methods affect the quality of bone histomorphology.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing best practices for human bone decalcification aimed at investigating the microscopy of bone and fracture healing. As such, the results of this study will be used as a standard protocol for decalcification of cranial bone from various aged decedents.

As part of a National Institute of Justice grant investigating cranial fracture healing, several decalcification techniques were explored to determine the amount of time for processing and the resulting quality of the healing bone histomorphology. Forensic histology laboratories often use Hydrochloric acid (HCL) because it provides the most expedient bone decalcification. However, there is evidence, that nitric acid and Ethylenediaminetetraacetic acid (EDTA) decalcification yield better results in non-human bone for the visibility of tissues, cells and molecules active in bone biology.<sup>1</sup> As most studies of cranial bone decalcification and healing use small animal models, this is an important first step to determine the methods best suited for human bone decalcification.

The sample is comprised of 50 samples from 23 individuals, ranging in age from 2 months to 77 years. Samples were fixed in 10% formalin for two to four weeks. Before decalcification, all bone sample dimensions and weights were recorded. Where possible, bone samples were cut into three sections for immersion in 10% EDTA, 5% nitric acid, and 7% HCL, and placed on an orbital shaker. Radiographic techniques were used to determine the degree of decalcification. In the initial radiographic inspection, settings were adjusted until radiopacity was consistent across the bone sample. These same settings were used in subsequent examinations until radiopacity was absent throughout the sample, indicating decalcification was complete. It was found that the radiographic technique varied primarily according to bone thickness and, secondarily to age. Thinner samples, such as infant cranial bone and thin areas of adult bone (e.g., pterion), required a setting of 40KVp to 48KVp and 0.9mAs to 1.8mAs. Older children and diploic bone required a setting of 50KVp and 2.5mAs.

After decalcification was complete, samples were rinsed in water for at least an hour to halt the decalcification process. Each sample was then cut with a scalpel and placed in a cassette for paraffin embedding and slide preparation. Three different histological stains were used, including Hemotoxylin and Eosin, Masson's Trichrome, and Alcian Blue Hematoxylin with an Orange G counterstain, resulting in 150 histological slides. Slides were assessed for the quality of cell, cell nuclei, and tissue visibility.

The results of the decalcification study show that for infants, juveniles, and adults HCL decalcification was the most rapid technique, followed by nitric acid and EDTA. For infants, HCL required 1 to 2 days, Nitric Acid required 2 to 5 days, and EDTA required 5 to 27 days for decalcification. For juveniles, HCL required 4 days, and nitric acid required 7 to 8 days for decalcification. Adult samples were more variable, requiring 2 to 4 days with HCL, 3 to 10 days with nitric acid, and 13 to 60 days with EDTA. EDTA decalcification times tended to increase with age; older aged adults required more decalcification days. A review of slide quality indicates that nitric acid and EDTA yield optimum results, with highly visible nuclei and greater stain color gradient contrast than HCL.

In conclusion, for high-quality slides produced relatively rapidly (1 to 10 days), nitric acid is the best decalcification technique for viewing histological structures related to bone. However, the gentle effects of EDTA chelation allowed samples to be used for immunostains and other special stains useful for investigating cells and tissues involved in bone healing.

*This project was supported by a National Institute of Justice grant.*

#### Reference(s):

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#### Decalcification Methods, Cranial Bone Histomorphology, Cranial Fracture Healing



### H147 Drugs and Homicide in Memphis and Shelby County: A Continued Epidemic of Violence—2014–2016

David W. Coates, MS\*, West Tennessee Regional Forensic Center, Memphis, TN 38105; Benjamin J. Figura, PhD, West Tennessee Regional Forensic Center, Memphis, TN 38105; Sherri L. Kacinko, PhD, Willow Grove, PA 19090; Kevin Jenkins, MD, West Tennessee Regional Forensic Center, Memphis, TN 38105; Paul V. Benson, MD, University of Tennessee Health Science Center/Shelby County Medical Examiner, Memphis, TN 38105

**Learning Overview:** After attending this presentation, attendees will understand the trends of select drug usage among homicide victims from 2014–2016 in Memphis, TN, and how it has changed since 1984–1986.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reporting on the detection of drugs in homicide victims and discussing statistical points of interest previously reported in the late 1980s.

The West Tennessee Regional Forensic Center performs autopsies on medicolegal, violent, or otherwise unnatural deaths in Memphis and Shelby County. Toxicological data is an important asset for determining the significance that drugs and alcohol have on the manner and cause of death, including homicide-related deaths. Specimens, including blood, urine, vitreous humor, liver, and gastric contents, are collected during autopsies and screened using common analytical toxicology methods, such as immunoassay, full-scan Gas Chromatography/Mass Spectrometry (GC/MS) or Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS). Positive findings are typically confirmed and quantified by GC/MS or Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). From 1980 to 1986, drug-related homicides increased according to a study by Harruff et al.<sup>1</sup> Exhaustive research showed that no current studies on drug-related homicides in Memphis and Shelby County have been published since 1988. This study reviewed toxicology findings from homicide cases examined at the West Tennessee Regional Forensic Center from 2014–2016. The purpose of this research was to determine the presence or absence of drugs in homicide victims and determine if any statistical trends were present.

Toxicology findings for known homicide cases from 2014–2016 were collected to build a data set in which decedent age, race, sex, and cause of death were tabulated. It was decided to focus this study on cases with positive results for THC, opiates, amphetamines, benzodiazepines, and ethanol. Previous research indicated that THC was not previously tested for but was included in this study due to its known widespread usage. Homicide victims were sorted according to their cause of death into the following major categories: gunshot wounds, blunt force trauma, stab wounds, delayed homicide, and other, and the frequency of positive results for each drug was recorded. Age as well as other demographics of homicide victims were categorized similarly to those used during the 1986 study to make comparisons. While previous research revealed that cocaine had risen to the most often-detected drug in homicide victims at the end of 1986, cocaine was found to be the third most-common toxicology result at the end of 2016. THC was found to be the most common positive toxicological result, accounting for 240 of the 460 total positive toxicological screens in the current study. Gunshot wounds remained the most prevalent cause of death associated with homicides in which drugs were detected, accounting for 83.7% of all drug-positive deaths. The most common victims of homicide, regardless of toxicology results, are African American males between the ages of 20 and 24 years. Review of these results indicated that illicit drug usage is widespread among homicide victims in Memphis, TN, and remains a common finding in homicide autopsies.

#### Reference(s):

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#### Drugs, Homicide, Toxicology



### **H148 Violence Against Vulnerable Persons: The Death of a Transgendered Individual in Mississippi**

*L.R. Funte, MD, Mississippi State Medical Examiner's Office, Pearl, MS 39208; Mark M. LeVaughn, MD\*, Mississippi State Medical Examiner's Office, Pearl, MS 39208; Brent Davis, MD, Mississippi State Medical Examiner's Office, Pearl, MS 39208; Anastasia Holobinko, PhD, Mississippi State Medical Examiner's Office, Pearl, MS 39208; Steven A. Symes, PhD, Mississippi State Medical Examiner's Office, Pearl, MS 39208*

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**THIS ABSTRACT WAS NOT PRESENTED.**



## H149 The Tale of an Operating Room Fire Fatality

Tasha Zemrus Greenberg, MD\*, Tarrant County Medical Examiner's Office, Fort Worth, TX 76104-4919

**Learning Overview:** After attending this presentation, attendees will understand the incidence, etiology, features, and consequences of Operating Room (OR) fires.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating medical examiners and forensic investigators about key features of OR fires, guiding the incident investigation and autopsy, and stressing the importance of interagency cooperation in the investigation.

On January 6, 2015, the fire department was contacted about an OR oxygen flash fire. Saline was used to douse the fire with minimal room damage. Fire investigators advised securing the OR until their inspection the next day. Upon arrival, the OR had been cleaned. A bed and pillow present showed burn marks. Items from the trash were retrieved. It was then reported that a patient was involved and had expired. The fire chief, hospital president, and police department were notified, and a detective was assigned.

At that time, the case was reported to the Tarrant County Medical Examiner's Office (TCME). The body was retrieved from a funeral home, and an autopsy was performed on January 8, 2015. On January 12, 2015, materials from the OR were received, including an orogastric tube, corrugated plastic tubing, oxygen face mask, blanket, plastic fragment, black strap, hospital gown, black covered foam, foam head rest, and two pillows with variable thermal damage and soot deposition in the face mask.

The patient's medical history included chronic obstructive pulmonary disease, amiodarone-related lung toxicity, hypertension, hyperlipidemia, diastolic heart failure, abdominal aortic aneurysm, gastroesophageal reflux disease, diverticulosis, and chronic anemia.

She presented to the hospital in December 2014 with abdominal pain due to diverticulitis with a small bowel obstruction and pelvic abscess and underwent small bowel resection with anastomosis on December 16, 2014. She developed respiratory failure requiring intubation, pneumonia with sepsis, acute kidney injury, and atrial fibrillation. She was extubated yet required 100% oxygen on BiPap, necessitating a tracheotomy in the OR.

The patient was wearing a Phillips® Respiration Fitlife full-face mask with a nasogastric tube coming out from under the mask with 100% oxygen flowing when Bovie electrocautery was used for the tracheotomy, causing the oxygen flowing through the mask to catch fire, leading to burns of the head, neck, right shoulder, and airway. It was not specified in the OR note if monopolar or bipolar diathermy was used. An emergent cricothyrotomy was performed; she failed extubation trials and expired later the same day after withdrawal of care.

There are multiple published reports of OR fires, estimating that 600-700 OR fires are reported each year, with many more unreported.<sup>1</sup> The most common situations include use of laser or cautery with an endotracheal tube, but any high oxygen concentration situation is a risk factor.

Recommendations by the American Society of Anesthesiologists Task Force on Operating Room Fires include use of diathermy, reduce build-up of oxygen beneath the surgical drape, and turn off oxygen one minute prior to using laser or cautery, with open communication between surgeon and anesthesiologist.<sup>2</sup> If a fire occurs, disconnect the oxygen supply, use a saline wash, and remove the cautery device. It is recommended to never use electrocautery when FiO<sub>2</sub> levels are greater than 50%.<sup>3</sup> CO<sub>2</sub> flooding of the surgical field can decrease the risk of fire in patients who cannot tolerate ventilation interruption.

The fire triad consists of fuel (tube/mask), oxidizing agent (oxygen), and ignition source (laser or electrocautery). OR fires are seen most commonly in head and neck surgeries and are less common now that less flammable anesthetic agents are used.

Findings at autopsy included thermal injuries of the head, neck and right shoulder, as well as the upper airway, in addition to emphysema, pneumonia, atherosclerotic cardiovascular disease with right ventricular dilatation, nephrosclerosis, diverticulosis, lymphadenopathy and splenomegaly. Toxicology was not contributory. The death was ruled an accident due to acute upper airway thermal injury due to medical equipment fire with chronic obstructive pulmonary disease, pneumonia, and hypertensive atherosclerotic cardiovascular disease with heart failure as contributory conditions.

The family filed a civil lawsuit against the surgeons, anesthesiologist, anesthesiology group, president, and vice president of the hospital as defined in the Texas Civil Practice and Remedies Code Section 74.001. The case was settled out of court for an undisclosed amount of monetary damages.

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### Operating Room Fire, Electrocautery, Fire Triad



### H150 The Botanical Survey in the Collection of Scientific Evidence for Reconstructing the Dynamics of Deaths From High Falls: A Case Report and Review of Literature

Isabella Aquila, MD\*, Institute of Legal Medicine, Catanzaro 88100, ITALY; Matteo A. Sacco, MD\*, Chair of Legal Medicine, University of Catanzaro, Catanzaro 88100, ITALY; Fabrizio Barbagallo, MD, Institute of Legal Medicine, Catanzaro, ITALY; Francesco Sicilia, MD\*, Institute of Legal Medicine, Catanzaro, ITALY; Vincenzo Rania, MD, Institute of Legal Medicine, Catanzaro, ITALY; Santo Gratteri, MD, Viale Europa, Germaneto, Catanzaro 88100, ITALY; Pietro Tarzia, MD, Institute of Legal Medicine, Catanzaro, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY

**Learning Overview:** After attending this presentation, attendees will understand the role of forensic botany in crime scene investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of botanical surveys in reconstructing the dynamics of death from high falls.

Scene investigation plays an important role in a crime. Often the crime scene is an open environment consisting of trees, plants, and shrubs. The forensic pathologist must compare the botanical elements with the evidence present on the victim.<sup>1</sup> Although the discovery of botanical elements on the scene is very common, to date forensic botany is a resource still underestimated. Investigators often fail to identify all elements and sometimes this evidence can be degraded or indistinguishable. For this purpose, DNA sequencing and biomolecular investigations become fundamental.<sup>2</sup> In literature, forensic botany was used to determine the path followed by the victim and to exclude other possible routes.<sup>3</sup> Forensic botany was crucial for demonstrating that the primary scene of a crime was falsified.<sup>4</sup> Sometimes, the crime scene and autopsy do not provide sufficient evidence. For example, in deaths from high falls, the forensic pathologist may have difficulty establishing the point of fall, the trajectory, the collision with obstacles, and the point of impact.

Reported here are two cases of death from high falls. The first case concerns a child who fell from the sixth floor of her home. The circumstantial data showed that the victim had fallen from the bathroom's window although there were some doubts about her suicide because of the presence of a gazebo that could be a possible way to escape from her house after a family quarrel. The girl was found on the ground near a gazebo that was completely destroyed. The gazebo was on the ground floor in the apartment of a neighboring family. About 1.5 meters from the gazebo, there was a separating hedge, more than two meters high. During the inspection, some botanical elements corresponding with the separating hedge were found on the victim's clothes and hair. The external examination excluded signs of a scuffle or violence. The botanical elements suggested an impact of the body on the hedge and subsequently a projection toward the gazebo with following breakage. According to the evidence on the scene, investigators established the mode of death due to suicide.

In the second case, an elder male was found on the ground, below a hill. The scene consisted of undergrowth vegetation. The head was resting on a blood-stained stone. Some shredded leaves were found in the victim's left hand, and some thorns were attached to the left sleeve of his sweater. Autopsy showed that the victim had widespread abrasions and bruises. A head injury was found, with petechiae (Wischnewsky Lesions) on the gastric mucosa. The autopsy showed no signs of scuffling and revealed that he died by frostbite because he was restrained due to head injury. There were doubts about the mode of death that were clarified by the botanical analysis of the point of fall and impact, confirming the hypothesis of a fall from height by accident.

Falls may be from a great height (more than ten meters) or from a medium height (less than ten meters). In the first case, there is a disproportion between the external injuries and the severity of organ lesions. In the second case, there are more external injuries.<sup>5</sup> In fatal falls, the evidence on the scene or autopsy findings are often insufficient to establish the dynamics. In the two cases reported, this study shows how the comparison between the data on the scene and the botanical findings on the corpse allow a reconstruction of the dynamics in relation to the external environment. The botanical analysis allowed calculation of the point of fall, the trajectory, and the point of impact precisely. These data were fundamental, together with other evidence collected, to determine the mode of death. For this reason, it is important, especially in cases of fatal falls in open environment, to: (1) evaluate the entry and exit ways of the area, (2) record the position of the corpse, (3) compare the botanical elements on the scene with the evidence on the clothes or victim, and (4) exclude signs of violence and other causes of death at autopsy.

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#### Forensic Science, Forensic Botany, Falls From Height



### H151 Deaths Due to Illegal Immigration: Always Drowning?

*Isabella Aquila, MD\**, Institute of Legal Medicine, Catanzaro 88100, ITALY; *Fabrizio Cordasco, MD\**, Università Magna Graecia CZ, Catanzaro, ITALY; *Francesco Sicilia, MD\**, Institute of Legal Medicine, Catanzaro, ITALY; *Santo Gratteri, MD*, Viale Europa, Germaneto, Catanzaro 88100, ITALY; *Matteo A. Sacco, MD*, Chair of Legal Medicine, University of Catanzaro, Catanzaro 88100, ITALY; *Pietrantonio Ricci, MD, PhD*, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY

**Learning Overview:** After attending this presentation, attendees will be able to describe the impact of differential diagnosis in suspected drownings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the role of autopsy and forensic investigations in landings of illegal immigrations.

The phenomenon of illegal immigration causes many victims every year. In most cases, the lack of safety measures on the boats increases the incidence of tragic accidents. Between 2002 and 2017, more than 300,000 immigrants died during illegal disembarkations on the Mediterranean Sea. This phenomenon still occurs on Ionian coasts.<sup>1</sup> In the majority of cases, the cause of death is drowning. Death also occurs due to pre-existing diseases, trauma, hypothermia, dehydration, and postpartum complications. The dangers include shipping incidents or crimes committed by smugglers against migrants who escape when they notice the authorities of the coast guard. The differential diagnosis between drowning and other causes of death is often complicated and requires multiple investigations. This issue is analyzed through a case of suspected drowning that occurred during an illegal landing.

**Case Report:** An unidentified Black youth, 14-16 years old, was found dead on a beach. The cause of death was unknown; the investigators suspected a drowning during an illegal landing. A judicial inspection and a radiological investigation (Computed Tomography/Mass Spectrometry (CT/MS)) were conducted. An autopsy, with histopathological investigation and search for diatoms, was performed. The data were compared and analyzed. Finally, a protocol provided by the International Criminal Police Organization (INTERPOL) for the identification of the corpse was activated.

**Results:** The external examination showed the presence of algae, sand, and piloerection with skin maceration. The autopsy revealed a wide fracture of the left femur. A massive pulmonary thrombus-embolus was found; other clots were found in the heart and were also within the structure of the papillary muscles and tendons, until the superior and inferior vena cava. A histopathological investigation showed the presence of diatoms within the lungs and other organs.

**Discussion:** Drowning is an asphyxia that is caused by the entry of water within the airway. Globally, deaths by drowning are estimated to be 370,000 per year.<sup>2</sup> Drowning may often occur in cases of illegal landings of migrants. In these cases, the forensic pathologist must exclude other causes of death before the diagnosis of drowning. Also, he must investigate signs of trauma, violence, or skin injuries on the body. In the case reported, the external examination didn't show typical signs of asphyxia. The autopsy did not show the presence of water in the stomach or bowel; the radiological investigations supported the autopsy findings. In addition, the histopathological investigation revealed the presence of diatoms within the lungs and other organs. This data could confirm the diagnosis of drowning. However, the autopsy revealed that the real cause of death was a pulmonary thromboembolism due to the femoral fracture. The diagnosis of drowning, therefore, was excluded. Finally, the dynamics were reconstructed: the boy was thrown into the sea and the trauma caused the left femur fracture; later he reached the shore, where he remained for about two days until he died of pulmonary thromboembolism. The case reported shows the importance of a careful interpretation of the data. The finding of diatoms is not always pathognomonic for drowning, and the diagnosis requires the exclusion of other possible causes. Therefore, a comparison of multiple forensic surveys is essential before diagnosis.

**Conclusions:** Illegal immigration is a major public issue. In case of illegal landings, forensic sciences are necessary to investigate cases of violence or deaths linked to the phenomenon. In these cases, the forensic pathologist performs a differential diagnosis with drowning. Many forensic surveys must be carried out before this diagnosis. Autopsy remains the gold standard for excluding other causes of death and to establish time and manner of the event.

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#### Forensic Science, Illegal Immigration, Drowning



### H152 An Unusual Case of Suicide Due to a Gas Mixture Poisoning

Isabella Aquila, MD\*, Institute of Legal Medicine, Catanzaro 88100, ITALY; Matteo A. Sacco, MD\*, Chair of Legal Medicine, University of Catanzaro, Catanzaro 88100, ITALY; Roberto Raffaele, BE\*, University Magna Graecia of Catanzaro, Crotona, ITALY; Santo Gratteri, MD, Viale Europa, Germaneto, Catanzaro 88100, ITALY; Luigi De Aloe, MD\*, Institute of Legal Medicine, Catanzaro, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Foggia 71100, ITALY

**Learning Overview:** After attending this presentation, attendees will understand the role of ethanethiol in acute poisoning by suicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing the chemical characteristics of the substance and its role in the putrefactive process.

The inhalation of volatile substances as a suicide method is rare, while the use of the same as abuse substances is quite common worldwide and is called Volatile Substance Abuse (VSA). In the majority of cases, the use of these substances as a suicide method is practiced by adult prisoners; the use of the same as abuse substances is more common in adolescents representing an important cause of mortality and morbidity in the young population. The most common types of volatile substances are aliphatic (butane, propane), aromatic (glues, lacquers, solvents), and halogenated hydrocarbons (spray paint, propellants). The methods of a substance's intake are: direct inhalation of the substance ("sniffing," inhalation by nose or the mouth), the inhalation through a cloth that is impregnated ("huffing"), and inhalation through a plastic bag. Liquefied Petroleum Gas (LPG) ( $\text{CH}_3 \text{CH}_2 \text{CH}_3 + \text{CH}_3 \text{CH}_2 \text{CH}_3$ ) is an odorless volatile substance and usually it is added with ethanethiol ( $\text{CH}_3 \text{CH}_2 \text{SH}$ ), a harmful gas with a bad odor (garlic). Ethanethiol has caused a neurological action on the central nervous system causing fatal respiratory depression. From the analysis of the literature, the main causes of death by acute poisoning are cardiac arrest (Sudden Sniffing Death Syndrome) and asphyxia, while the main causes of death due to chronic abuse are cardiomyopathy, myocardial infarction, and central nervous system toxicity.<sup>1,2</sup> Postmortem analysis on biological fluids and tissues is performed by the gas chromatography method.

A Belarusian boy (weight 100kg, height 185cm) was found dead in his home (ambient temperature: 26°C). The boy was in the kitchen in a supine position with a black plastic bag wrapped around his head with a hole from which he passed a gas hose connected to a domestic LPG tank. A judicial inspection was conducted to exclude any signs of a scuffle or break-in. At home, antipsychotic drugs were found. The analysis of the witnesses among the family members showed that the boy was suffering from psychosis with hallucinations and a history of drug addiction. The psychological autopsy showed his sister's suicide from height fall. The boy and his sister were both adopted by an Italian family. An autopsy was performed. The corpse appeared in a putrefaction state (12 hours after death). The hypostasis were abundant in red-bluish color at the back, chest, and neck. The lungs showed edema with anthracosis areas. The heart revealed small sub-epicardic petechiae. The buccal cavity showed a mucous hyperemia. A toxicological and histopathological investigation was carried out. The organs' analysis was limited by the presence of putrefaction, in particular on the brain and pancreas. The toxicological analysis showed an increase of blood glucose and the presence of antipsychotic drugs due to pharmacological therapy. Butane and propane concentrations were found in the blood, bile, and adipose tissue. Ethanethium's concentration was detected in peripheral blood and urine.

The analysis of this case shows that the gas mixtures used as suicide methods can be lethal even at low concentrations. In the case of LPG, the chemical analysis shows that the most lethal or responsible gas in the genesis of death is ethanethiol. The lethal effect of ethanethiol can be due to the -SH chemical group, which has a high biological toxicity with potential affinity for hemoglobin. The lethal effect is also due to the mixture of gas with the known toxicity of butane and propane.<sup>3</sup> From the forensic point of view, in the analysis of the postmortem interval, it is crucial to underline that, in cases of gas mixture poisoning, the presence of ethanethiol speeds up the putrefaction due to the -SH chemical group for the composition of sulfuric methemoglobin responsible for the putrefactive process.

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#### Forensic Science, Ethanethiol, Acute Poisoning



### **H153 A Multidisciplinary Forensic Approach in the Analysis of Skeletal Remains: A Chronology of Injuries and Dynamics of Events**

*Sara Sablone, MD, Institute of Legal Medicine, Bari 70124, ITALY; Antonio De Donno, PhD, Bari 70124, ITALY; Chiara Lauretti, Institute of Legal Medicine, Bari, ITALY; Iliaria Santoiemma\*, Bari 70124, ITALY; Francesco Introna, MD, Dim Sezione Di Medicina Legale, Bari 70124, ITALY*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### H154 Spleen Attenuation and Routine Measurements With Regard to Cause of Death and Estimation of Spleen Weight: A Study on Postmortem Computed Tomography (PMCT)

Vasiliki Chatzaraki, MD\*, Institute of Forensic Medicine Zurich, Zurich, Europe CH-8057, SWITZERLAND; Wolf Schweitzer\*, Universitat Zurich, Zurich CH-8057, SWITZERLAND; Michael Thali, MD, Universitat Zurich, Zurich CH-8057, SWITZERLAND; Garyfalia Ampanozi, MD, Institute of Forensic Medicine, Zurich 8057, SWITZERLAND

**Learning Overview:** The goal of this presentation is to show how spleen characteristics as interpreted by imaging tools like PMCT can contribute in the determination of a cause of death and predict accurately the actual spleen weight before an autopsy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by stating that various peri-mortem conditions, such as hospitalization, long or short agonal interval, and cause of death may influence organ findings on imaging. Additionally, by the increasing usage of postmortem imaging as a supplemental tool to autopsy worldwide, organ measurements and weight estimation must be validated.

**Objectives:** The spleen is the largest lymphatic organ of the human body. Its size, weight, and macroscopic features are of great forensic importance as acute organ responses or chronic diseases can be reflected in the spleen's appearance during an autopsy and may contribute to the determination of a cause of death.<sup>1</sup> PMCT is an invaluable supplement to traditional forensic autopsy.<sup>2,3</sup> The first goal of the current study was to investigate spleen attenuation on PMCT with respect to different causes of death, macroscopic spleen findings during autopsy, the presence of Contrast Agent (CA) rests, and hospitalization prior to death. The second goal was to evaluate the utility of PMCT to perform routine measurements for estimating the actual spleen weight measured during autopsy.

**Materials and Methods:** The autopsy protocols and PMCT data of all cases with PMCT and full forensic autopsy from 2015 to 2017 were reviewed retrospectively. Spleen attenuation in Hounsfield Units (HU), Width (W), Thickness (T) in cm and axial maximal Surface (S) in cm<sup>2</sup> were measured on PMCT. The cause of death, macroscopic spleen findings (paleness, contraction, congestion), and Spleen Weight (SW) during autopsy, whether death occurred in hospital or not, and the usage of CA prior to death were listed retrospectively for each case. Cases <18 years old, postmortem interval >4 days, asplenia, organ explantation, severe spleen injuries, and cases with artifacts or gas within the spleen on PMCT were excluded. Statistical analysis was performed to investigate first the spleen attenuation values among different causes of death, macroscopic findings, hospital and non-hospital deaths, and CA usage, and second, to assess the utility of PMCT for estimation of the actual spleen weight.

**Results:** One thousand thirty-five cases were included in this study, 972 of them for exploring spleen attenuation and 1,026 for evaluating spleen measurements for the prediction of spleen weight. Spleen attenuation average was 49.51HU ±6.54HU. Spleen attenuation was significantly higher for cases with CA rests on PMCT (52.05HU±9.22HU), contrary to cases without CA (49.3HU±6.24HU) and significantly lower for hospital-deaths (48.44HU±8.82HU) compared to non-hospital deaths (49.77HU±5.79HU). Spleen attenuation did not differ significantly among the different macroscopic spleen findings. Sixteen different cause-of-death groups were identified, and spleen attenuation was significantly lower for multiple organ failure (43.71HU±11.2HU) compared to fatal hemorrhage (50.01HU±6.42HU), intoxication (50.05HU±5.40HU), strangulation-asphyxia (50.83HU±5.41HU), hypothermia (52.44HU±4.31HU), and metabolic disorders (52.57HU±7.39HU). Spleen W was 8.60cm±1.78cm, T: 4.10cm±1.08cm and S: 26.63 cm<sup>2</sup>±10.72cm<sup>2</sup>. The average SW was 188.69g±108g. There were strong positive correlations among W-T-S and SW, and the correlations were expressed as linear regressions with equations for predicting SW based on W, T, and S on PMCT. There were no statistical differences between calculated SW and actual SW. The regression equations overestimate smaller SW and underestimate larger SW.

**Conclusion:** Spleen attenuation on PMCT differs significantly among several causes of death. CA usage and prior hospitalization must always be taken in account during spleen radiological findings' interpretation as CA increases the organ attenuation and intravenous fluid therapy during hospitalization decreases attenuation causing hemodilution. PMCT can accurately estimate SW based on spleen measurements on axial PMCT slices.

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#### Spleen Attenuation, Spleen Weight, Postmortem Computed Tomography



### H155 “Enlightened”: The Effect of Training Forensic Pathology Residents With LED-Enhanced Point-and-Shoot Cameras for Forensic Macro Photography

Valeria Hofer\*, University of Zurich, Zuerich CH-8057, SWITZERLAND; Michael Thali, MD\*, Universitat Zurich, Zurich CH-8057, SWITZERLAND; Rosa M. Martinez, MD\*, Universitat Zurich, Zurich CH-8057, SWITZERLAND; Wolf Schweitzer\*, Universitat Zurich, Zurich CH-8057, SWITZERLAND

**Learning Overview:** After attending this presentation, attendees will better understand that training forensic pathology residents in the use of properly set up point-and-shoot cameras can yield satisfying results in forensic macro photography.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a very low-cost modification of a point-and-shoot camera with an added camera-mounted Light-Emitting Diode (LED) lamp and by demonstrating how training amateurs with respect to forensic macro photography can generate satisfying results.

**Introduction:** With increasing exposure of our forensic pathology residents to event scene and death scene photography as well as for clinical forensic medicine deployments, where forensic scientists lack capacity for photography support, the need to burden them with technical extra training becomes more acute. With a team of Forensic Pathology Residents (FPR) of 10–15 people, operative advantages arise from buying affordable point-and-shoot rather than Digital Single Lens Reflex (DSLR) cameras. While the hard restrictions for macro photography (aperture, exposure time, ISO number) create an actual operation space without added light, adding light by mounting a simple LED torchlight using a 3D-printed lamp Mount (LEDMNT) seemed to massively increase image quality. This is an intervention study comparing the image quality before and after training a number of FPR without other formal photography training.

**Methods and Materials:** This study used four test objects and compared the image quality achieved by six FPR before and after specific individual training in which they were instructed in optimal settings for ISO number, aperture, exposure time for 10-15 minutes each, and after additionally adding the added LEDMNT. For each image, a score S was awarded based on image characteristics read out from the Exchangeable Image Format (EXIF) meta-data contained in the digital files and based on subjective impression of image focus (1 rater), that would result in any S theoretically ranging from 0 to 40.

**Results:** For each step along the training, scores increased: (1)  $S=14\pm4$ ; (2)  $S=21\pm2$ ; and (3)  $S=30\pm4$ , with statistically significant differences comparing (2) to (1), (3) to (2), and (3) to (1). The images also showed subjective aspects of improvement; most notably, they were more focused and appeared to contain less noise.

**Discussion:** While training FPR alone (without adding LEDMNT) already increased the image quality both objectively and subjectively, adding extra light (LEDMNT) added another significant quality improvement. Bringing very affordable camera-mounted extra light in macro photography therefore must be regarded as a significant step beyond knowledge, insight, and training.

#### Virtopsy, Forensic Macro-Photography, In-House Training



### **H156 Multiplanar Reconstruction (MPR) of Antemortem Computed Tomography (CT) Images to Visualize Sphenoidal Sinus and Cranial Base Morphology for Identification Purposes**

*Carlos J. Zambrano, PhD\**, Office of the Chief Medical Examiner, Oklahoma City, OK 73105; *Angela Berg, RN*, Office of the Chief Medical Examiner, Tulsa, OK 74107; *Marc Allen Harrison, MD*, Office of the Chief Medical Examiner, Oklahoma City, OK 73105; *Eric Duval, DO*, Office of the Chief Medical Examiner, Oklahoma City, OK 73117; *Inas Z. Yacoub, MD*, Office of the Chief Medical Examiner, Oklahoma City, OK 73105

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**Learning Overview:** After attending this presentation, attendees will understand how to process an antemortem CT image series into a 2D projected image using Multiplanar Reconstruction (MPR) that can be used, in conjunction with or in lieu of conventional antemortem radiographs, to establish positive identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a simple methodology to project an antemortem CT series that can be easily compared to a postmortem radiograph or postmortem CT. Additionally, this methodology can be used to provide an antemortem radiograph of a missing person that could be used to compare to unidentified skeletal remains.

Establishing positive identification via scientific means is a primary objective of the medicolegal death investigation. Identity can be determined quickly via fingerprint comparison if the decedent has prints on record. In cases in which there are no prints on file or the decedent is in advanced stages of decomposition or completely skeletonized, the use of other methods such as DNA or radiographic comparisons is necessary. Radiographic comparisons are a likely means for positive identification because they obviate the practical and logistic limitations of other methods and are often readily available in many instances.

Clinical medicine has steadily increased the use of CT imaging since their introduction resulting in the possibility of using antemortem CT data for identification purposes. Although CT scout films may be taken during a clinical exam, the image quality and/or view may not provide sufficient points of concordance to conclude a positive identification when comparing them to postmortem radiographs. A solution to this is to utilize software that allows MPR of the CT data to create a projected image to facilitate comparison to postmortem radiographs or CT. This methodology is particularly useful when dealing with skeletal remains and could provide an additional approach for identification via radiological comparisons from antemortem CT images of missing persons to unidentified skeletal remains within the National Missing and Unidentified Persons database (NamUs).

Obtaining antemortem CT scans during a death investigation is a common occurrence; however, technical knowledge on how best to utilize this data varies greatly between personnel and agencies conducting a death investigation. This presentation will: (1) present free and cost-effective software options to create an MPR of antemortem CT data, (2) provide a basic review and steps on how to create a 2D projection from the MPR, (3) present three examples of 2D projections that achieve positive identification by using sphenoidal sinus and cranial base morphology, (4) discuss future research utilizing MPR of antemortem CT data, and (5) the utility of creating projections of missing persons in NamUs to possibly aid in the identification of unidentified individuals. The implementation MPR-projected images from an antemortem CT series will provide forensic anthropologists, radiologists, odontologists, and pathologists another avenue for the positive identification of remains.

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#### **Forensic Radiology, Positive Identification, Computed Tomography Scan Projection**



### H157 3D Anatomical Model Acquisition and Reproduction of Human Organs: Which Perspectives for Forensic Pathology?

Antonio Guajana, Palermo 90149, ITALY; Francesco Di Paola, University of Palermo, Palermo, ITALY; Marco Arrigo, Palermo 90145, ITALY; Stefania Zerbo, MD, Via Del Vespro, 127, Palermo 90100, ITALY; Elvira Ventura Spagnolo, MD\*, University of Palermo, Palermo 90127, ITALY; Gennaro Baldino, Patti, ITALY; Daniele Daricello, MD, University of Palermo, Palermo 90127, ITALY; Dario Saguto, MD, Palermo, ITALY; Mariella Farella, MD, Palermo, ITALY; Giosue' Lo Bosco, PhD, Università degli studi di Palermo, Palermo 90123, ITALY; Fabio Bucchieri, MD, Palermo, ITALY; Francesco Cappello, Palermo, ITALY; Antonina Argo, Palermo 90100, ITALY

**Learning Overview:** The goal of this presentation is to draw attention to obtaining virtual 3D models of human organs, which can help to visualize gross and micro-anatomical features from a 360° perspective that could be used as strong evidence during cross examination in trial.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by drawing attention to obtaining virtual 3D models of human organs.

This technology was initiated to support university medical students in their learning activities during a human anatomy laboratory. In literature, there are several examples of virtual reality models and systems developed to teach about human anatomy and some of them are an excellent reproduction of the reality. However, these supports do not address one very important aspect: touch and handle the reality.<sup>1,2</sup>

The use of such technology has turned out to be an excellent innovative instrument for the scientific community, not only for learning purposes, but making it possible to visualize gross and micro-anatomical features from a 360° perspective that could be used as strong evidence during cross-examination in trial.

This system has a built-in database of 3D human organ models. This database was used to map the models for augmented reality recognition steps to anchor the educational tags and to reproduce real human organs with a 3D printer. Furthermore, to achieve proper virtual representation of the models, this study has conducted an accurate digital acquisition with latest generation instruments and, consequently, the data processing. The process begun on the physical model is defined as reverse engineering, and digital resolution up to 0.1mm was realized using a 3D portable scanning system with a structured light flash bulb, permitting a highly detailed digital model to be produced. Non-invasive technology has proved particularly suitable in relation to the physical characteristics of the real organs, including the dimensions, the complexity of the external surfaces, and the constraints on manipulation.

The application to a real case of the hanging of a young female victim allowed the guarantee of an augmented reality and 3D reconstruction of the anatomical structures of the neck for permanent visualization and peer-review evaluation in court, worthy of mention as tools for cross examination.

#### Reference(s):

1. Argo A., Arrigo M., Bucchieri F., et al. Augmented Reality Gamification for Human Anatomy [Article in press].
2. Hackett M., Proctor M. Three-Dimensional Display Technologies for Anatomical Education: A Literature Review. *Journal of Science Education and Technology* 25(4), 641–654 (2016).

**Forensic Pathology, 3D Anatomical Human Model, Augmented Reality-Hanging**





### H158 The Value of Postmortem Computed Tomography (PMCT) in the Examination of Advanced Decomposed Bodies

Alessandro Santurro, MD\*, Sapienza, University of Rome, Rome 00161, ITALY; Gaia Cartocci, Rome, ITALY; Matteo Scopetti, MD, Sapienza University of Rome, Rome, ITALY; Vittorio Gatto, MD, Sapienza University of Rome, Rome 00185, ITALY; Mariantonia Di Sanzo, MD, Rome, ITALY; Raffaele La Russa, MD, Rome, ITALY

**Learning Overview:** After attending this presentation, attendees will better understand the importance of PMCT in the examination of advanced decomposed bodies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of PMCT as a useful tool in detecting postmortem gas, even in very small amounts, and by allowing better discrimination of natural biological changes from pathologic processes in advanced decomposed bodies.

Decomposed bodies represent a real challenge for forensic pathologists because putrefaction processes can impressively alter the appearance of the bodies. In this context, PMCT represents an accurate procedure, allowing one to distinguish between normal postmortem changes and pathologic findings (i.e., between putrefactive gas present in normal decompositional processes and pathologic gas collections contributing to death, such as air embolism, pneumothorax, or pneumoperitoneum).

Between May 2013 and November 2016, ten forensic cases (8 males, 2 females) were selected from those admitted to this study's Forensic Unit by the local inquiring authorities to ascertain the cause of death. The Postmortem Interval (PMI) ranged from 7 to 887 days. All the bodies were PMCT-scanned prior to autopsy according to a standardized scanning protocol. PMCT data were transferred to a workstation for post-processing image reconstruction and were finally analyzed using a viewing software.

The internal putrefactive state was determined using the Radiological Alteration Index (RAI), determined by PMCT in seven selected sites, including the major vessels (left innominate vena and abdominal aorta), selected bones (vertebra L3), selected organs (heart cavities, liver parenchyma and vessels, and kidney parenchyma), and subcutaneous tissues and muscles (subcutaneous pectoral tissues), according to a standardized protocol (grade of gas present: 0, I, II, or III).<sup>1</sup> After PMCT scans, a complete conventional autopsy of each body was performed. The Grade of External Putrefaction (GEP) was assigned by the forensic pathologist during the external examination of the bodies, according to a standardized classification (beginning, moderate, advanced, major, mummified).<sup>2</sup> Causes of death were further investigated by histological, immunohistochemical, and toxicological examinations.

As expected, the PMCT image evaluation and the internal state analysis of putrefaction revealed that, in the selected seven sites, the RAI was >70 in all bodies, meaning advanced decomposed status. The gas grade was higher in correspondence of the major vessels, heart cavities, vertebra L3, heart cavities, and subcutaneous pectoral tissues. Coherently, the GEP assessment at the cadaveric external examination revealed the presence of transformative phenomena from "major" to "mummified" in all the examined bodies.

This study offers an overview of the common findings in advanced decomposition that may dramatically alter a body's appearance and consequently add more difficulty in determining the cause of death, significantly increasing the risk of bias and leading to misinterpretation of the autopsy findings. Radiological imaging by PMCT as an adjunct to conventional autopsy in advanced decomposed bodies has been proven to be a useful tool in detecting postmortem gas, even in very small amounts. Putrefaction can be described in postmortem imaging as gas accumulation within vascular system, body cavities, parenchyma, and soft tissues.

Conclusively, the radiological investigation offers an indisputable support to conventional autopsy in cases of decomposed bodies, increasing its performance and allowing the study of findings that would otherwise be difficult to identify. For these reasons, the correct interpretation of PMCT data is fundamental to avoid the misunderstanding of instrumental evidence and to facilitate the differential diagnosis between natural decomposition and pathological processes.

#### Reference(s):

1. Egger C., Vaucher P., Doenz F., Palmiere C., Mangin P., Grabherr S. Development and Validation of a Postmortem Radiological Alteration Index: The RA-Index. *Int J Legal Med.* 2012;126(4):559-66.
2. Maujean G., Vacher P., Bagur J., Guinet T., Malicier D. Forensic Autopsy of Human Decomposed Bodies as a Valuable Tool for Prevention: A French Regional Study. *Am J Forensic Med Pathol.* 2016;37(4):270-274.

#### Postmortem Computed Tomography, Forensic Radiology, Advanced Decomposed Bodies



### **H159 An Interesting and Timely Dental Identification in a Uniquely Posed Decomposed Female Using Premortem Computed Tomography (CT) Images and Postmortem Radiographs**

*Tasha Zemrus Greenberg, MD\*, Tarrant County Medical Examiner's Office, Fort Worth, TX 76104-4919; Roger D. Metcalf, DDS, JD, Tarrant County, Fort Worth, TX 76179*

**Learning Overview:** After attending this presentation, attendees will appreciate an interesting case of dental identification performed at the Tarrant County Medical Examiner's Office (TCME).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating medical examiners and forensic odontologists about the utility of postmortem dental radiograph comparison with premortem CT scan images and the importance of having a qualified forensic odontologist as part of an identification team to aid in the process of identification in difficult cases in which fingerprints cannot be obtained.

The Tarrant County Medical Examiner's Office in Fort Worth, TX, possesses a state-of-the-art Human Identification Laboratory whose director is a board-certified forensic odontologist who holds a diploma in Forensic Human Identification from the Faculty of Forensic and Legal Medicine of the Royal College of Physicians in London. The Human Identification team also consists of a board-certified forensic anthropologist and two fingerprint examiners. In 2016, the office identified approximately 89% of 751 unidentified decedents within 24 hours of arrival by fingerprint analysis. Approximately 2% of cases at TCME require dental analysis for identification.

On July 18, 2018, a decomposed female was found in an apartment residence. She had last been known to be alive in June 2018 by her mother who requested a welfare check when she was unable to contact her. The decedent was found standing at the kitchen sink with her upper body slumped into the sink. The interior of the apartment was extremely hot (104.9°F), though there was an air conditioner present. There were no illegal drugs, prescription medications, alcohol, or paraphernalia found in the apartment.

The decedent was known to be a 55-year-old White female with a history of schizophrenia, hypertension, mental retardation, and gastroesophageal reflux. She was non-compliant with her medications and had multiple psychiatric hospital admissions.

At the time of the postmortem examination, the body was cold following refrigeration. Rigor mortis was absent, having passed in the small and large muscles. Livor mortis was not readily discernible. The body was malodorous with near complete mummification and moist decomposition of the left upper extremity, sloughing of hair and scalp with exposure of portions of the skull, degloving of the left hand, exposure of the bones of the left third through fifth fingers, absence of the left ear, dessication of the eyes, and purge fluid present in the external nares, oral cavity, and anus. Maggots were present up to one-quarter inch in length as well as fly eggs and small millipede-like insects.

Record searches were conducted based on the tentative identity. Database searches included Tarrant County Sheriff's Office (TCSO) database and the Texas Crime Information Center/National Crime Information Center (TCIC/NCIC). Possible records were located with TCSO, the Texas Department of Public Safety (TXDPS), and the Federal Bureau of Information (FBI). However, postmortem fingerprints were unable to be obtained due to the state of decomposition.

Antemortem radiographs were requested from the treating hospital and a Compact Disc (CD) of images of her head was received on July 19, 2018. Comparisons were made using a transverse (axial) plane postmortem X-ray image of the skull and appropriate "slices" from the antemortem CT scan, and a definitive identification based on dental features was made the same day.

This case of dental identification illustrates the utility of comparing postmortem radiographs with premortem CT scans for identification purposes, as well as the importance of having a qualified forensic odontologist available for these unique cases in which fingerprints are unable to be used.

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#### **Dental Identification, CT Image and Radiograph Comparison, Forensic Odontology**



### **H160 Postmortem Computed Tomography (PMCT) Investigation in Cases of Suspected Infanticide**

*Nivia A.M. Matias, MD\**, Civil Police of Federal District, Brasília, Distrito Federal 70673-000, BRAZIL; *Sara Anieli Costa Braz Fonseca, MD, PCDF*, Brasília, BRAZIL

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**THIS ABSTRACT WAS NOT PRESENTED.**



### H161 Fatal Nitrous Oxide Intoxication—Suicide or Accident?

Serge Schneider, PhD, Laboratoire National de Santé, Dudelange 3555, LUXEMBOURG; Martine Schaul, MD, Laboratoire National de Santé, Dudelange 3555, LUXEMBOURG; Thorsten Schwark, MD\*, Laboratoire National de Santé, Dudelange 3555, LUXEMBOURG

**Learning Overview:** After attending this presentation, attendees will: (1) have been provided with an overview of Nitrous Oxide (N<sub>2</sub>O) abuse and its—potentially fatal—risks, and (2) be acquainted with the toxicological analysis of N<sub>2</sub>O.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sensitizing crime scene investigators, forensic pathologists, and forensic toxicologists to the dangers of N<sub>2</sub>O abuse, thus improving crime scene work, autopsy procedures, and subsequent toxicological analysis.

N<sub>2</sub>O (“laughing gas”) is an odorless gas that is used as a (weak) anesthetic in clinical settings. Due to its dissociative and euphoric effects, and its easy availability, it is also widely abused in a recreational context. The gas is usually inhaled (e. g., out of filled balloons) and due to the short half-life of the substance, the effects wear off quickly. The toxicity of N<sub>2</sub>O is low; however, if inhalation occurs in a limited space, displacement of oxygen may lead to hypoxia and, ultimately, death. In chronic users, N<sub>2</sub>O is known to interact with vitamin B12 containing enzyme systems, and N<sub>2</sub>O abuse may lead to hematological, immunological, and neurological symptoms. Even though some abuse-related fatalities have been reported, deaths caused by N<sub>2</sub>O inhalation are rare events.

Here, the unexpected death of an 18-year-old boy is described. The deceased was found by his mother fully dressed in a prone position in his parents’ house. A 25L plastic bag, connected to a whipped cream steel siphon using a plastic tube, was pulled over his head and fixed around his neck. The police secured five used cream charger capsules originally containing 7.5g of N<sub>2</sub>O each on a table close to the deceased; one additional empty capsule was found in the capsule holder applied to the empty siphon.

According to his parents, the boy had been in a depressed mood in the days prior to his death since his girlfriend had left him but had not expressed any suicidal thoughts. A school friend reported that the deceased had told him he had previously inhaled “laughing gas.”

Autopsy and histology results were unremarkable except for scattered conjunctival petechiae, congestion of the inner organs, and brain and lung edema. Toxicological analysis using static headspace Gas Chromatography/Mass Spectrometry (GC/MS) analysis proved the presence of N<sub>2</sub>O in lung tissue and blood; a screening for alcohol and licit and illicit drugs was negative. Considering autopsy and toxicological findings, asphyxiation caused by oxygen depletion and N<sub>2</sub>O inhalation was ascertained as the cause of death.

As previously described by Bäckström et al., N<sub>2</sub>O concentrations in the inspired air of 25 % suffice to depress the sensation of dyspnea in the person inhaling the gas, thus leading to sudden unconsciousness due to hypoxia.<sup>1</sup> It is assumed that the administered N<sub>2</sub>O volume in this case was at least 3.8L (7.5g N<sub>2</sub>O equal 3.8L at 0°C); considering the five additional capsules found at the scene, it may even have been significantly more (up to 45g N<sub>2</sub>O, equaling approx. 23L at 0°C). By introducing the gas into the reduced remaining space of the plastic bag, N<sub>2</sub>O levels that are sufficient to explain a sudden unconsciousness and subsequent incapacity to act can easily be provoked. Further investigations in the peer group of the deceased confirmed that he had abusively used N<sub>2</sub>O before so that the manner of death in this case was ruled an accident rather than a suicide.

The presented report illustrates a rare case of fatal N<sub>2</sub>O inhalation, and clearly emphasizes the hazards accompanying “laughing gas” abuse.

#### Reference(s):

<sup>1</sup>. Bäckström B., Johansson B., Eriksson A. (2015) Death From Nitrous Oxide. *J Forensic Sci* 60: 1662-1665. doi: 10.1111/1556-4029.12879.

#### Nitrous Oxide, Hypoxia, Substance Abuse



### H162 Accidental Corrosive Acid Poisoning in an Infant: A Case Report

*Manoj Bhusaheb Parchake, MD\*, Seth GS Medical College & KEM Hospital, Mumbai, Maharashtra 400012, INDIA*

**Learning Overview:** After attending this presentation, attendees will understand the pattern of corrosive injuries on the mucous surface of the mouth and skin of an infant and its interpretation. Attendees will also learn the causes behind accidental corrosive acid poisoning.

**Impact on the Forensic Science Community:** This presentation will impact the forensics science community by serving in the diagnosis and treatment of corrosive poisoning in an infant. This presentation will help the forensic expert rule out child abuse cases and implement preventive measures.

Corrosive substances may cause severe to serious injuries of the upper gastrointestinal tract, and the poisoning can even result in death. Accidental ingestion of caustic agents continues to be a major concern for pediatric emergency department clinicians. Corrosive agent ingestions are seen most frequently in young children between one and three years of age, is very rare in infants, and can cause severe acute injury and long-term complications. A 3-month-old male infant was brought to the hospital with a history of blackening of the tongue, injuries over his body, and difficulty breathing after taking some medicine. The pediatrician suspected child abuse and hence sent a call to forensic medicine. After evaluating the infant and detailed history from the relative, it was confirmed that the blackish color was a corrosive injury on the mucosal surface of the mouth, lip, and chest and a reddish color on neck folds and the back. After an ultrasonography, severe epiglottis edema was noticed, and no esophageal or upper digestive tract perforation. On blood investigation, severe acidosis was observed, and the infant was immediately put on a ventilator and treatment began. Aspirated fluid was sent for chemical analysis.

The blackish color of the corrosion and normal urine, favored sulfuric acid poisoning. Phenol corrosive injury is brownish in color with greenish or even black color urine on exposure to air, while nitric acid has is a yellowish color corrosion. On treatment, gastric lavage and emetics were contraindicated and nothing was given orally for two to three days. Broad spectrum antibiotics and IV fluids were given to the infant. On further history it was found that concentrated sulfuric acid was kept in medicine bottle and given by mistake by the infant's aunt. The pattern of corrosive acid injury was blackish in color and the injuries over the chest and neck folds were due to the dribbling of the acidulated fluid from the angle of the mouth. It was confirmed by the forensic science laboratory that sulfuric acid was present in the medicine bottle. Regarding the manner of injuries, it was likely to be accidental as it is common practiced in the slums of Mumbai to keep cheap locally available sulfuric acid in a soda bottle or cold drink bottle or any bottle that is available, such as a medicine bottle.

#### Accidental, Corrosive, Poisoning



## H163 Homicide by Intramuscular Cyanide Injection: Investigation and Adjudication

Megan R. Hall, BA\*, Eastern Virginia Medical School, Norfolk, VA 23507; Wendy M. Gunther, MD, Office of the Chief Medical Examiner, Tidewater District, Norfolk, VA 23510-1046

**Learning Overview:** After attending this presentation, attendees will: (1) be familiar with the presentation of cyanide poisoning, (2) be aware of historical examples of cyanide poisoning, and (3) have learned about the adjudication of a case of homicide by intramuscular cyanide poisoning.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through increased competence in recognizing the signs and symptoms of cyanide poisoning and understanding the adjudication of a case of a homicide by cyanide injection.

Fatalities from cyanide are relatively rare. Most cyanide fatalities involve ingestion by mouth or inhalation of cyanide gas.<sup>1</sup> Ingestion of cyanide has been used in homicides, such as the unsolved case of the “Tylenol® murders” in Chicago, in which several people died from unknowingly ingesting acetaminophen laced with cyanide. Its gaseous form has been used in genocide, as was seen with the use of Zyklon B in Nazi Germany. Suicide by cyanide gas or ingestion is more common than homicide; both are infrequent in the United States, as cyanide is difficult to obtain.<sup>2</sup> Historical records show that cyanide was ingested in pill form by Nazi officers to commit suicide; it was mixed with Kool-Aid® in the Jonestown mass suicide; and the computer scientist Alan Turing laced an apple with cyanide when persecuted for his sexual orientation. Fatalities by cyanide injection, particularly homicidal, are rarely reported.<sup>2,3</sup> This report presents the investigation, autopsy, and adjudication of a case of homicide by cyanide injection.

A young woman returning from work, on exiting her car, was attacked by a man who had been hiding in her bushes. He stabbed her in the left buttock with a needle, injecting something, and ran away. The victim cried out to her mother in agony and collapsed. Emergency medical services were activated and noted that the victim was unresponsive, with decorticate posturing, and with a gaze that was fixed to the left. Communication with her mother was hindered by a language barrier; seizure was initially suspected.

On emergent transport to the local hospital, she was in distress; hypotensive, with progressively falling blood pressure; in acute respiratory failure; and in metabolic acidosis, with a profound anion gap. Global hypoxic-ischemic encephalopathy on Computed Tomography (CT) scan developed within hours; death ensued 20 hours after the assault. Hospital toxicology on plasma drawn about four hours after the assault identified cyanide at a level of 4.053mg/L (reported normal range, non-smoker, less than 0.025mg/L; smoker, average 0.41mg/L; death, associated with values greater than 3mg/L).

Forensic autopsy identified a dermal puncture on the left buttock, found no competing cause of death, and no pregnancy. The postmortem toxicology report confirmed the presence of cyanide.

In the meantime, police developed information implicating the decedent’s ex-boyfriend, who was also the father of the decedent’s 2-year-old child; they were in the middle of a custody battle. He was also a suspect in an assault on the same victim two and one-half months earlier, during which the assailant, who attacked her when she exited her vehicle, tossed a cupful of chemical liquid into her face.

Approximately 15 months after her death, trial began. Evidence submitted by prosecution included security footage from outside the decedent’s home that captured the attack, a description of the assailant by the victim’s mother, forensic evidence from the defendant’s computer, including multiple web searches such as “what if cyanide gets injected?” and “how many mg of cyanide will kill you?,” and documentation that he purchased a hypodermic needle from an internet seller. The hypodermic needle was delivered to a pizza parlor he frequented, with testimony from the employees that he witnessed retrieving it. Prosecution was unable to document how or where the defendant might have purchased cyanide.

Defense focused on a claim that the defendant could not have attacked the decedent because he was 2.5 hours away, in a different city, as illustrated by phone records. However, the defendant’s phone, although remaining in that city, was not used for outgoing phone calls or texts for a period of 22 hours (from 3 hours prior to the attack until 19 hours afterward).

The defendant was found guilty of first degree murder; sentencing is pending.

### Reference(s):

1. Chin R., Calderon Y. Acute Cyanide Poisoning: A Case Report. *The Journal of Emergency Medicine* 2000; 18: 441-445
2. Prieto I., Pujol I., Santiuste C., et al. Acute Cyanide Poisoning by Subcutaneous Injection. *Emergency Medicine Journal* 2005; 22:389-390.
3. Abeyasinghe N.L., Perera H.J.M., et al. Case Report—Death by Subcutaneous Injection of Cyanide in Sri Lanka. *Journal of Forensic and Legal Medicine* 2011; 18: 182-183

### Intramuscular Cyanide Injection, Homicide by Cyanide Injection, Forensic Sciences



### H164 Validation of a Urine Drug Screen Assay in the Postmortem Setting

Emily Wolak, DO\*, Albuquerque, NM 87111; Sarah Lathrop, DVM, PhD, Albuquerque, NM 87111; Monique Dodd, PharmD, Albuquerque, NM 87112; Hannah A. Kastenbaum, MD, University of New Mexico, Albuquerque, NM 87131

**Learning Overview:** This goal of this presentation is to review the utility of a Urine Drug Screen (UDS) for the purposes of triaging autopsies, discussing if this method is a valid substitution for blood testing, and if it has potential to maximize operational efficiency. As advised by National Association of Medical Examiners (NAME) guidelines, individuals should undergo a complete autopsy when “death is by apparent intoxication.” Consequently, the high number of drug-related deaths across the country adds financial burden to many offices. There may be a place for point-of-care UDS assays in the postmortem setting where they can serve as a source of preliminary information regarding the presence of potentially fatal substances. In this way, the assay can guide further testing and management of the case. If a urine assay is found to be a valid and inexpensive substitute for blood testing in the postmortem setting, the operational efficiency implications are significant.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a framework for the use of a UDS assay as a potential substitute for blood testing in certain cases.

**Methods:** This study tested the performance of a UDS assay compared with toxicological analysis of postmortem blood as the gold standard for 87 cases requiring full autopsy at the New Mexico Office of the Medical Investigator. Ten common drug classes of abuse were analyzed: methamphetamine, amphetamine, cocaine, opioids, barbiturates, Tricyclic Antidepressants (TCAs), methadone, Tetrahydrocannabinol (THC), benzodiazepines, and Phencyclidine (PCP). Blood toxicology was tested through National Medical Services (NMS). The case pathologist interpreted the results of the UDS, and these interpretations and photographs of the card were documented. Once the toxicology reports from NMS were complete, the final interpretation of the urine screen by the case pathologist was compared to the NMS blood toxicology results. For each of the ten classes of drugs tested for by the UDS, the numbers of concordant and discordant pairs were calculated. McNemar’s test for correlated proportions was used, with a *p*-value of 0.05 or less considered statistically significant. Sensitivity and specificity were also calculated.

**Results:** For the 87 cases tested, the postmortem blood was analyzed with either the NMS basic toxicology panel (68 cases) or an expanded panel (19 cases). Discrepancies between the UDS and blood Gas Chromatography/Mass Spectrometry (GC/MS) were classified as either “triage-relevant” or “triage-irrelevant” for purposes of cause and manner of death investigation. For example, false negative cocaine on the UDS was considered triage-relevant, whereas false negative THC was considered triage-irrelevant. All false positives were considered to be triage-irrelevant. Three decedents had partial results due to assay error (failure of urine to move up the well), and so data was only included for those drug classes with results present. By McNemar’s test, only the opioid drug category had a statistically significant difference in results discordance between the UDS and NMS blood toxicology results. The remaining nine classes of drugs showed no statistically significant difference between NMS and UDS results. Forty-six of 87 cases (53%) had a discordance of some type between the UDS and NMS results; however, 16 of those (35%) were “excused” due to testing differences, or if the drug was present in the urine only (and not in the blood). Nineteen of the 87 cases (22%) had a triage-relevant discordance, such as a false negative detection of a potentially fatal drug.

**Conclusion:** Of the ten classes of drugs analyzed, only opioids showed a statistically significant discordance. For this study’s purposes, this is a triage-relevant discrepancy as opioid false negatives can inaccurately alter the course of the death investigation. However, it should be noted that this discrepancy was not due to assay error, because the majority of false negative opioids on the urine screen were due to fentanyl, which is not detected by this assay. Most importantly, a lack of statistical significance does not necessarily imply clinical irrelevance, if even a small number of false negative results could adversely impact downstream clinical decision making. It was concluded that while the UDS screen is consistent with blood GC/MS findings for most classes of drugs, it is at the discretion of the forensic pathologist to incorporate all investigative information when utilizing the UDS as a potential triage tool.

*This study was funded by the Forensic Sciences Foundation Acorn grant.*

#### Assay, Drug Screen, Autopsy



### H165 An Immunohistochemical Study of Central Nervous System (CNS) Damage in Two Drug Abusers (Synthetic Cannabinoids, Synthetic Cathinones, and Phenethylamine Derivatives)

Mio Takayama, PhD\*, Fukuoka University, Fukuoka 814-0180, JAPAN; Masayuki Kashiwagi, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN; Aya Matsusue, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN; Brian Joseph Waters, MS, Fukuoka University, Department of Forensic Medicine, Fukuoka 814-0180, JAPAN; Kenji Hara, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN; Natsuki Ikematsu, BPharm, Fukuoka University, Fukuoka 814-0180, JAPAN; Shin-ich Kubo, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN

**Learning Overview:** After attending this presentation, attendees will understand the immunohistochemical changes that occur in the brains of abusers of novel psychoactive substances.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing the abuse of novel psychoactive substances, such as synthetic cannabinoids, synthetic cathinones, and phenethyl amine derivatives, and how they affect the brains of users.

**Introduction:** Drug abuse remains a major social problem in society today. In forensic autopsy cases, drugs such as methamphetamine, amphetamine, and illegal herbal products are occasionally detected. This study examines CNS damage immunohistochemically in drug abusers.

**Materials and Methods:** Two cases were selected from cases autopsied at Fukuoka University within 48 hours of the postmortem interval, where synthetic cannabinoids, synthetic cathinones, or phenethylamine derivatives were detected. Case 1 involved a man in his early 30s. Toxicological analysis revealed the presence of 6-APB, 6-MAPB, DL-466,  $\alpha$ -PHP, and mepirapim in the blood and urine. His cause of death was acute drug intoxication. Case 2 also involved a man in his early 30s. Toxicological analysis revealed methamphetamine, amphetamine, DL-466,  $\alpha$ -PHP, N-fluoropentyl-AB-PINACA, and 5-Fluoro-AMB in the blood or urine. His cause of death was asphyxia by choking on regurgitated stomach contents. The hippocampus and cerebellum were collected from formalin-fixed forensic autopsy brains from each case. Histochemical stainings were observed with Hematoxylineosin (HE) and Luxol Fast Blue (LFB). Immunohistochemical stainings were performed using antibodies against MAP2 (1: 200, Abcam plc, UK), Glucose Transporter 5 (GLUT5) (1: 200, Abcam plc, UK), GFAP (1: 1000, Abcam plc, UK), and Iba-1 (1: 350, Abcam plc, UK) with the EnVision™ Detection System/HRP according to the manufacturer's instructions. MAP2 and GLUT5 were for neurons, GFAP was for astrocytes, and Iba-1 was for microglia.

**Results:** The histochemical stainings from Case 1 showed morphological changes in the neurons from Dentate Gyrus (DG) to CA3 in the hippocampus. Fewer granule cells in the granular layer of the cerebellar cortex were observed. Immunohistochemically, the immunoreactivity of the pyramidal cells lessened from CA2 to CA1 with MAP2, and in DG with GLUT5. An increase of GFAP-positive astrocytes in DG and Iba-1-positive microglia overall in the hippocampus were observed. In the cerebellar cortex, the immunoreactivity of granule cells in the granular layer lessened with MAP2. GFAP-positive astrocytes increased in the granular layer, and the fibers of the astrocytes were rosary-shaped in the cerebellum. In Case 2, histochemical stainings showed no significant findings in the hippocampus and cerebellum. Immunohistochemically, the MAP2-positive pyramidal cells from CA3 to Subiculum (SUB), and GLUT5-positive pyramidal cells in SUB and from DG to CA3 were observed. GFAP-positive astrocytes increased in the pyramidal cell layer. Immunoreactivity of MAP2-positive and GLUT5-positive were shown in Purkinje cells and molecular cells.

**Conclusion:** The hippocampus and cerebellum in the brains of two drug users who abused synthetic cannabinoids, synthetic cathinones, or phenethylamine derivatives were examined immunohistochemically. Some morphological and immunoreactive changes were observed in the neurons, astrocytes, and microglia. To understand more clearly about the pathological findings of the relationship between drug abuse and damage to the brain, more cases and other immunohistochemical stainings need to be examined.

#### Neuropathology, Drug Abuser, Immunohistochemistry





### H166 Filicide-Feticide-Suicide: An Unusual Variant of Triadic Death

*Nilesh K. Tumram, MD\*, Department of Forensic Medicine and Toxicology, Nagpur, Maharashtra 440013, INDIA*

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**Learning Overview:** After attending this presentation, attendees will be able to evaluate the triadic death, which may be a distinct type of homicide-suicide-feticide of the killing of children by a parent (filicide-suicide) and the killing of the fetus inside womb of mother (feticide). The terms “maternal filicide” or “paternal filicide” are used respectively when the perpetrator is the mother or the father of the victim. A rare case of maternal filicide-feticide is reported, in which the mother poisoned her son and then herself consumed the same poison, causing the live fetus to die because of the poisoning of the mother.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by creating awareness of another rare form of filicide-feticide-suicide, presenting a case highlighting the fact that such a manner is rarely been seen.

Murder-suicide, homicide-suicide, and dyadic death all refer to an incident in which a homicide is committed, followed by the perpetrator’s suicide almost immediately or soon after the homicide. Homicide of a child by its parents is called filicide. Homicide-suicides are relatively uncommon and vary from region to region. However, on review of literature, there is no mention of a case in which there is a killing of a child by the mother by giving poisonous material in milk and similarly consuming the same poison for self-killing.

In the present case, there was stress caused by repeated domestic quarrels to the pregnant mother having one child. In a fit of rage, the mother mixed insecticide granules in the milk of her son and made him drink it. Then the mother drank the remaining portion of the milk. The son became ill in a few minutes and laid unconscious with his mother, who also fell unconscious after some time. The mother knew that consuming the poison herself could harm her fetus too, but still committed the act, leading to the death of the fetus inside her womb. Thus, there was an unusual variant of filicide- feticide-suicide that caused a triad of death that can aptly be termed as triadic death.

This study presents a case of triadic death in which the method of killing and suicide was poisoning, and the unborn fetus died due to the consequences of self-poisoning by the young woman. This presentation will also discuss the importance of having knowledge of such a manner of death while dealing with medicolegal cases.

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#### **Filicide-Feticide, Suicide, Triadic Death**



### H167 The Evolution of the Opioid Crisis in Cuyahoga County, Ohio, From 2012 to 2016

Vaishali S. Deo, MD\*, Case Western Reserve University School of Medicine, Cleveland, OH 44106; Thomas P. Gilson, MD, Cuyahoga County Medical Examiner's Office, Cleveland, OH 44106

**Learning Overview:** The goal of this presentation is to characterize fentanyl overdose victims of 2016 and compare them with heroin-associated fatalities of 2012.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the role of the medical examiner in addressing multiple aspects of the opioid crisis by serving as a public health data source and a bridge between different stakeholders (law enforcement, public health officials, medical community, etc.) to combat the opioid epidemic.

Fentanyl and its analogs have emerged since 2014 as the leading driver of opioid mortality in the United States. This is in contrast to the scenario that was seen immediately prior when heroin came to prominence after the initial wave of overdose deaths associated with prescription Opioid Pain Relievers (OPR). It has been assumed that the drug abuse population has remained the same throughout, but there is little evidence characterizing the fentanyl overdose victims. The objective of this study is to look at the deaths associated with fentanyl in 2016 and compare the characteristics of 2016 fentanyl (with or without heroin) overdose deaths with heroin-associated fatalities in 2012 in Cuyahoga County, OH. This study also looks at the three-year prescription data of cases of 2016 fentanyl overdose deaths in Cuyahoga County for prescription of OPR and evidence of "doctor shopping" and compares it with the prescription data of 2013 heroin-associated fatalities. The retrospective prescription data of 2013 was more complete than that of 2012 as full medical examiner access to the prescription drug monitoring program was initially delayed until then.

Socio-demographic characteristics of cases of fentanyl overdose deaths in 2016 were compared with those of heroin fatalities in 2012. Data were abstracted from Cuyahoga County Medical Examiner case files. The prescription data of 2016 fentanyl mortalities were retrieved from the records of the Ohio Automated Rx Reporting System (OARRS), the prescription drug monitoring program for the state of Ohio. Doctor shopping was defined as five or more prescribers of a controlled substance (e.g., OPR) in a 12-month period.

The number of fatalities in Cuyahoga County increased from 160 heroin-related deaths in 2012 to 417 fentanyl-related deaths in 2016. However, no significant differences in socio-demographic characteristics were seen among cases of 2016 fentanyl and 2012 heroin overdose deaths. In both the cohorts, the overdose victims were predominantly 35 to 64 years of age (60%–64%), White (84%) males living in an urban setting (44%–45%). Analysis of OARRS data of 2016 fentanyl overdose deaths showed 70% of the cases having records on file in OARRS, which was similar to the 2013 OARRS records of heroin fatalities. However, doctor shopping showed a decline from 36% in 2013 to approximately 22% in 2016.

This study reports a substantial increase in fentanyl-related deaths in 2016 compared to heroin deaths in 2012. Baseline demographics between both cohorts are similar. OPR drugs remain a current factor in the drug crisis. This study emphasizes the role of the medical examiner in providing data for opioid-associated mortality, support, and collaboration with public health partners.

#### Fentanyl, Heroin, Mortality



### **H168 Drug and Alcohol Trends in Fatally Injured Drivers in the State of Maryland (2007–2018)**

*Gruschenka Mojica Sanchez, MD\**, Office of the Chief Medical Examiner, Baltimore, MD 21223; *Rebecca Jufer Phipps, PhD*, State of MD, Office of the Chief Medical Examiner, Baltimore, MD 21223; *David R. Fowler, MD*, Office of the Chief Medical Examiner, Baltimore, MD 21223

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**THIS ABSTRACT WAS NOT PRESENTED.**



### H169 Aqueous Fluid as a Viable Substitute for Vitreous Fluid in Postmortem Chemistry Analysis

*Daniel C. Butler, MD\**, Medical University of South Carolina, Charleston, SC 29403; *Cynthia A. Schandl, MD, PhD*, Medical University of South Carolina, Charleston, SC 29425; *S. Erin Presnell, MD*, Medical University of South Carolina, Department of Pathology, Charleston, SC 29425

**Learning Overview:** The goal of this presentation is to describe the correlation between vitreous and aqueous analytes and the use of aqueous fluid in the pediatric autopsy setting.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that in certain circumstances, particularly pediatric autopsies, vitreous fluid is not sampled due to the risk of introducing hemorrhagic artifact to the globe of the eye. In the current study, aqueous fluid is shown to significantly correlate with vitreous fluid. If predictable differences are considered, aqueous fluid may be sampled in place of vitreous fluid without the risk of introducing artifact.

**Background:** Vitreous fluid analytes can be a valuable investigative tool in postmortem forensic examinations. While clinical practice relies on blood for analyte analysis, postmortem chemistry is complicated by factors such as continued cell metabolism and autolysis with cell membrane degeneration, making it unsuitable in the forensic setting. As a preserved site with low cell counts, vitreous fluid is somewhat isolated from these changes, allowing for an evaluation of antemortem chemistry. However, in pediatric forensic autopsies where head trauma is present, vitreous fluid sampling is discouraged if eye removal is warranted to evaluate for retinal hemorrhages so as not to introduce hemorrhagic artifact. Whereas vitreous humor is the predominant fluid of the eye, filling the space between the lens and retina, the anterior chamber between the iris and cornea contains a smaller volume of aqueous fluid, which, when sampled, does not introduce hemorrhagic artifact to the posterior chamber as it is physically separated from the retina. Procurement and analysis of aqueous fluid was proposed as a substitute for vitreous fluid.

**Methods:** Vitreous and aqueous fluid were sampled concordantly from 22 pediatric decedents (median age=0.67 years) and 42 adult decedents (median age=45 years) at the Medical and Forensic Autopsy Division of the Department of Pathology and Laboratory Medicine at the Medical University of South Carolina between 2011 and 2018. Aqueous fluid was sampled with a 25-gauge needle and vitreous fluid was sampled with an 18-gauge needle at the time of autopsy. Sodium (Na), potassium (K), chloride (Cl), urea nitrogen (UN), creatinine (Cr), and glucose (Glc) aqueous and vitreous concentrations were compared to determine correlation and reliable difference. Samples above or below the detectible limit were excluded from the study.

**Results:** Spearman's rank-order analysis detected significant correlations between pediatric, adult, and combined pediatric/adult samples, respectively:  $\rho=0.89$ ,  $\rho=0.93$ , and  $\rho=0.84$  between vitreous Na and aqueous Na;  $\rho=0.96$ ,  $\rho=0.93$ , and  $\rho=0.93$  between vitreous K and aqueous K;  $\rho=0.53$ ,  $\rho=0.81$ , and  $\rho=0.79$  between vitreous Cl and aqueous Cl;  $\rho=0.91$ ,  $\rho=0.93$ , and  $\rho=0.95$  between vitreous UN and aqueous UN;  $\rho=0.79$ ,  $\rho=0.85$ , and  $\rho=0.86$  between vitreous Cr and aqueous C; and  $\rho=0.81$ ,  $\rho=0.77$ , and  $\rho=0.77$  between vitreous Glc and aqueous Glc.

Simple linear regression was utilized to assess associations in aqueous analytes based on corresponding vitreous analyte values in pediatric, adult, and combined pediatric/adult samples, respectively. For Na, aqueous values were 23%, 8%, and 12% higher than vitreous Na on average; aqueous K was 6%, 8%, and 5% higher than vitreous K on average; aqueous Cl was 34%, 15%, and 18% lower than vitreous Cl on average; aqueous UN was 3%, 2%, and 2% lower than vitreous UN on average; aqueous Cr was 31%, 13%, and 11% lower than vitreous Cr on average; and aqueous Glc was 11%, 29%, and 16% lower than vitreous Glc on average.

**Conclusion:** Significant correlation exists between vitreous and aqueous analytes with predictable differences.

Taking into account these differences, aqueous fluid analysis of Na, K, Cl, Cr, UN, and Glc concentrations would be a viable substitute for vitreous in the postmortem setting; however, a more robust study population with further consideration of postmortem interval, age, gender, race, and cause of death is required to determine the difference between aqueous and vitreous analyte values with a higher degree of specificity.

#### Forensic Pathology, Vitreous Fluid, Aqueous Fluid



### H170 The Frequency of Cannabinoids in a Medical Examiner Population

*Caitlin N. Markey, Lansing Catholic High School, Lansing, MI 48912; Michael A. Markey, MD\*, Sparrow Forensic Pathology, Lansing, MI 48912; Patrick A. Hansma, DO, Sparrow Forensic Pathology, Lansing, MI 48912; Luke R. Vogelsberg, Sparrow Forensic Pathology, Lansing, MI 48912*

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**Learning Overview:** After attending the presentation, attendees will: (1) have a better understanding of how often cannabinoids are detected in various types of death that fall within the jurisdiction of the medical examiner's office, (2) be able to compare this frequency with the reported frequency of cannabis (marijuana) use, and (3) be able to compare the frequency in which cannabinoids are detected with the frequency in which ethanol is detected in these cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic community by documenting the current use of cannabis in the medical examiner population and suggesting trends that the forensic community may expect to see with increasing legalization of cannabis use in the United States.

Within the past several years, many states have legalized the use of cannabis for medicinal use. There has been an ever-increasing push to expand the legalization of cannabis for recreational use. Eight states and the District of Columbia have current laws legalizing cannabis for recreational use. Proponents of marijuana legalization profess its safety, but with the expected trend of legalizing marijuana use, the question of its potential impact on mortality must be considered.

Recently published data from The Substance Abuse and Mental Health Services Administration indicates that self-reported current use of marijuana (cannabis), defined as use within the past month, in the United States for the year 2016 was 8.9% of the population (those 12 years of age or older). The same agency reported that for the state of Michigan, the estimated current use of marijuana by the same population was 10.45% (averaged for the years 2014 to 2016). For comparison, the self-reported current use of ethanol in the United States for the year 2016 was 50.7%.

The electronic database of a forensic pathology practice that provides medical examiner services to several counties in mid-Michigan was queried for death investigations that included a postmortem examination and toxicology testing for the period of January 1, 2017, to May 31, 2018. During this time, 929 death investigations included a postmortem examination, and, of these, 884 had toxicology testing also performed. The cases evaluated included 193 natural deaths, 424 accidental deaths, 168 suicides, and 40 homicides.

In all cases reviewed, 21.7% had a postmortem toxicology screen (blood, urine, or other) that was positive for cannabinoids (THC and/or THC-COOH), significantly higher than the reported frequency of "current use" in the United States and Michigan. By type of case, the breakdown of a positive toxicology screen for cannabinoids was as follows: 45% of homicides, 25.7% of accident drug intoxications, 25% of suicides, 21.9% of drivers in motor vehicle accidents, 21.5% of drownings, 16.6% of natural deaths, and 15.4% of passengers in motor vehicle fatalities.

By comparison, in all cases reviewed, 23.5% had a postmortem toxicology screen positive for ethanol, significantly lower than the reported "current use" in the United States.

The results of this study indicate that cannabinoids are present at a higher-than-expected frequencies in the studied medical examiner population when compared to baseline reported marijuana use (using current data based on self-reported).

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#### Cannabinoids, Marijuana, Medical Examiner



### H171 Generalized Organomegaly and the Use of Performance-Enhancing Substances Identified Following Sudden Death

Stephanie Diu, BA\*, Rutgers Robert Wood Johnson Medical School, Piscataway Township, NJ 08854; Jennifer L. Hammers, DO, Pittsburgh, PA 15219

**Learning Overview:** The goal of this presentation is to highlight the death of a 43-year-old Caucasian male due to hypertensive and atherosclerotic cardiovascular disease, in which the use of body-building enhancers was a significant contributing factor.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the potential effect of anabolic steroids on organ structure and how these changes can lead to organomegaly and cardiovascular damage.

**Introduction:** Performance-enhancing drugs are pharmacologic agents that professional and recreational athletes use to improve competitive performance or physical appearance. These drugs include anabolic-androgenic steroids, human growth hormone, and blood boosters such as erythropoietin.<sup>1</sup> There are significant gaps in scientific knowledge regarding the adverse health effects of performance-enhancing drugs. Anabolic-androgenic steroids have been associated with increased systolic and diastolic blood pressure, left ventricular hypertrophy, cardiomegaly, and acute myocardial infarction.<sup>2,3</sup> There is evidence that mortality is significantly increased in steroid abusers over non-abusing athletes.<sup>2</sup> Case reports typically feature a male poly-drug user with drug toxicity being the most common cause of death and extensive cardiovascular disease being a notable feature.<sup>4</sup> Per this study's research, this is the first case report linking generalized organomegaly with use of performance-enhancing drugs.

**Materials and Methods:** This case involved a well-developed (6 feet 4 inches, 252 pounds), trim, muscular 43-year-old Caucasian male who collapsed suddenly while exercising on a treadmill. He had been complaining of headaches with facial reddening over the weeks before death. He was known to use body-enhancing substances.

**Results:** Postmortem examination revealed generalized organomegaly and extensive cardiovascular disease. The pancreas was uniformly markedly enlarged and weighed 450 grams. The kidneys weighed more than 300 grams each, the liver weighed 3,350 grams, and the spleen weighed 450 grams. The decedent was found to have cardiac hypertrophy (630 grams), concentric left ventricular hypertrophy (2.1cm), and right ventricular hypertrophy (0.6cm). There was atherosclerosis with slight (50%) multifocal luminal impingement in the basilar artery and Circle of Willis arteries, as well as marked three-vessel coronary artery atherosclerotic stenosis (75%–95%). Further examination revealed a remote myocardial infarct in the left lateral ventricular wall (3cm x 3cm x 2.1cm), and an adjacent recent myocardial infarct in the posterior lateral left ventricular wall (5cm x 3cm x 2.1 cm).

**Discussion:** Generalized organomegaly could be an adverse effect caused by body-enhancing substances and warrants further investigation in future studies. The potential dangers of performance-enhancing drugs are likely underreported because anabolic-androgenic steroids are not routinely screened during autopsy, as testing for performance-enhancing substances can be difficult and expensive.<sup>1</sup> In addition, it is often difficult to obtain a complete and accurate history regarding the use of these substances. This case emphasizes the need for further investigation regarding the prevalence of performance-enhancing drug use and the mechanisms by which these drugs cause adverse effects.

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#### Body-Building Enhancers, Organomegaly, Anabolic Steroids



### H172 Three Deaths in Tarrant County, Texas, Related to the Use of 25H-NBOMe, 25B-NBOMe, or 25I-NBOMe

Tasha Zemrus Greenberg, MD\*, Tarrant County Medical Examiner's Office, Fort Worth, TX 76104-4919; Robert D. Johnson, PhD, Tarrant County Medical Examiner's Office, Fort Worth, TX 76104; Richard C. Fries, DO, Fort Worth, TX 76104; Susan J. Roe, MD, Tarrant County Medical Examiner's Office, Fort Worth, TX 76104

**Learning Overview:** After attending this presentation, attendees will understand the clinical presentations of NBOMe intoxication after review of three cases from the Tarrant County Medical Examiner's Office (TCME).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating medical examiners and toxicologists about behaviors associated with NBOMe drugs and by helping identify cases in which these drugs should be considered.

The recent increase in the use of NBOMe compounds, variants of the 2C-X series of psychoactive phenethylamines with significantly greater potency, have resulted in numerous deaths around the world as well as Emergency Department (ED) admissions by individuals suffering from erratic and dangerous behavior.<sup>1</sup> The NBOMe compounds act via the 5-HT<sub>2A</sub> receptors, leading to intense hallucinations with actions that can be self-directed or focused on other individuals. The effects last between four and ten hours, and users report euphoria, mental/physical stimulation, feelings of empathy, alterations in consciousness, and hallucinations. ED staff may be faced with individuals who are very difficult to control. Medical examiners may receive a decedent with a history of bizarre behavior prior to death.

Case 1 involved a 20-year-old male who became unresponsive after taking a capsule. Emergency Medical Services (EMS) found him in convulsions. Two other young men at the scene also became unresponsive. All were transported to the hospital for suspected overdoses. The first man expired. The friends recovered and told a detective that they had ingested gel caps filled with a white powder called NBOMe. Postmortem toxicology testing was positive for 25B-NBOMe at 47.9ng/mL and 25H-NBOMe at 0.030ng/mL in hospital blood, as well as THC, oxycodone, hydrocodone, and hydromorphone in hospital urine. The death was attributed to NBOMe toxicity.

Case 2 involved a 22-year-old male found lying supine on the living room floor of a home that was in extensive disarray. There was copious blood throughout the residence and surrounding the decedent. A window in the dining room was broken from the inside with blood and hair on it. The decedent's girlfriend revealed that four days prior, they had taken "hits of acid" with two male acquaintances; the decedent took four doses. After several hours, he became aggressive and demanded the males leave. They returned hours later and found him lying "bloody" and unresponsive on the floor, although they did not report it. The girlfriend was not at the residence; however, she presented to an ED with an orbital fracture and concussion, sustained in an altercation with the decedent.

At autopsy, he had multiple injuries, including sharp force injury of the right ear with a portion of the pinna absent, as well as reduced livor mortis and organ pallor consistent with acute blood loss. Initial toxicological examination on biological specimens was negative. Case circumstances directed subsequent testing for novel psychoactive drugs. 25H-NBOMe [2-(2,5-dimethoxyphenyl)-N-(2-methoxybenzyl) ethanamine, monohydrochloride] and 25B-NBOMe [4-bromo-2,5-dimethoxy-N-[(2-methoxyphenyl)methyl]-benzeneethanamine] were identified. The death was attributed to exsanguination due to sharp force injury of the ear, with drugs considered a contributory factor to his behavior.

Case 3 involved a 20-year-old male seen on surveillance video at a car dealership jumping on and smashing a windshield, then driving his vehicle through a glass window. When confronted by police, he behaved erratically and ignored commands. One officer discharged his Taser<sup>®</sup>, and another discharged his firearm, both striking the decedent. He died due to gunshot wounds to the neck, chest, and abdomen. Toxicology testing was positive for 25I-NBOMe [2-(4-iodo-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine] at 0.76ng/mL and THC at 3.1ng/mL in femoral blood, and 25H-NBOMe in urine. The drugs were considered a contributory factor to his behavior.

The extreme behavior demonstrated in two of these cases is not unusual, as agitation and aggression are often reported in cases involving the NBOMe compounds. Extreme behaviors may be dose dependent. Some individuals may not measure the drugs correctly and/or may not know what they are ingesting.

Due to the lack of literature available for these specific NBOMe compounds, it is difficult to interpret the concentrations seen in these cases. As the prevalence of these drugs continues to rise, it is important to publicize the dangerous and often lethal side effects of these compounds.

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#### NBOMe Fatalities, Psychoactive Phenethylamines, Hallucinogens



### H173 Accidental Overdose Deaths Involving Fentanyl and the Growing Trend of Counterfeit Prescription Pills in New Mexico

Jolee T. Suddock, DO\*, Department of Pathology, University of New Mexico, Albuquerque, NM 87131; Sarah Lathrop, DVM, PhD, Albuquerque, NM 87111; Garon Bodor, MS, University of New Mexico, Office of the Medical Investigator, Sandia Park, NM 87047; Matthew D. Cain, MD, Office of the Medical Investigator, Albuquerque, NM 87102

**Learning Overview:** After attending this presentation, attendees will understand trends in fentanyl-related overdose deaths in New Mexico (NM) and describe the prevalence of counterfeit prescription pills in relation to demographics and region.

**Impact on the Forensic Science Community:** This study will impact the forensic science community by providing a better understanding of at-risk populations in relation to fentanyl overdose death and by providing analysis that will aid in the construction and implementation of targeted prevention strategies.

**Background:** Fentanyl is a potent synthetic opioid known to be illegally manufactured and used recreationally. It has become increasingly prevalent in the United States and has significantly contributed to the opioid epidemic. Various news sources have cited overdoses in which individuals are unaware of fentanyl contamination. Although counterfeit prescription pills mixed with fentanyl have been reported in other states, there has only recently been a spike in these cases in NM. NM has encountered several cases in which apparent prescription pills were found at the scene, yet toxicology reports have detected only a high concentration of fentanyl. To combat the evolving fentanyl epidemic, it is vital to characterize these cases to develop targeted interventions.

**Purpose:** The purpose of this study is to evaluate which counterfeit prescription pills are most commonly encountered, determine whether differences exist in concentrations of fentanyl in pill versus intravenous death, and determine if regional clusters exist.

**Methods:** Data for drug-related deaths, confirmed by epidemiology and forensic pathologists, were retrieved from the NM Office of the Medical Investigator database from 2014 to July 2018. All accidental deaths involving fentanyl were evaluated for the presence of pills found at the scene and a history of prescription abuse. Deaths involving probable injection of an illicit drug, such as heroin and/or methamphetamine, were used as a comparison for the concentrations against individuals taking counterfeit pills but were not the focus of this study. Cases in which fentanyl patches were observed, or the decedent was legitimately prescribed fentanyl, were excluded. In addition to statistical analysis, these cases were geocoded using Google® Fusion Tables.

Analysis was performed using Statistical Analysis Software (SAS). Categorical variables were compared using either a chi-square test or a Fisher exact test if an expected cell count was less than five. Continuous variables were compared using a Wilcoxon rank-sum test or a Kruskal-Wallis for multiple comparisons. P values of 0.05 or less were considered statistically significant.

**Results:** There were 158 cases of accidental overdoses involving fentanyl in NM. Of those cases, 55 involved prescription fentanyl patches (27 prescribed, 23 non-prescribed, and 5 had an unknown prescription status) and 29 involved suspected counterfeit pills. Decedents were primarily White non-Hispanic and Hispanic males ( $p < 0.02$ , mean ages 38.1 and 38.4, respectively).

The cases involving suspected counterfeit pills were chosen based on the presence of pills at the scene or in which a substantiated history of illicit prescription pill purchase was available. Oxycodone was the most commonly encountered (14 cases), followed by alprazolam (10 cases), morphine (3 cases), and oxycontin (1 case). One case involved an unidentified pill. Fentanyl concentrations for cases of suspected pill overdose were not significantly different than those of intravenous drug use. The earliest case appeared in late 2014 and involved an oxycodone addict who died of a remarkably high fentanyl concentration. Afterward, the majority of cases appeared in 2016 and onward, with a spike in 2018 ( $p < 0.0001$ ). While this data is preliminary, there have been at least three cases in which the individual thought they were consuming oxycodone, but the pills actually contained fentanyl.

These cases were plotted by year and by drug using Google® Fusion Tables. A majority occurred near Albuquerque, NM, likely with population concentration a significant contributing factor. However, alprazolam and oxycodone mimics were noted to appear in southern NM in 2017 and are clustered near interstates. These data are being shared with the Drug Enforcement Agency and discussions are ongoing.

#### Fentanyl, Overdose, Counterfeit Pills



## H174 A Five-Year Retrospective Study of Drug Abuse Deaths in Maryland (2013–2017)

Ayomide Oludoyi, BSc\*, University of Maryland, Baltimore, Baltimore, MD 21201; Sarah J. Sides, BS\*, University of Maryland, Baltimore, Baltimore, MD 21201; Alyssa Dean, MS\*, University of Maryland, Baltimore, Baltimore, MD 21230; Allison C. Klein, BA, University of Maryland Baltimore, Graduate School, Baltimore, MD 21201; David R. Fowler, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223; Mary G. Ripple, MD, Baltimore, MD 21223; Ling Li, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223

**Learning Overview:** After attending this presentation, attendees will better understand the epidemiological characteristics and current trend of drug abuse deaths in America.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the urgent need for more aggressive regulatory, educational, and treatment measures to address the tremendous increase in fatal drug abuse deaths, especially fentanyl-related deaths.

Deaths involving drug use have been rapidly rising over the years, especially with the introduction of fentanyl into the drug community. In 1992, drug deaths accounted for 249 cases in the State of Maryland, while 25 years later, drug-related deaths were 2,333 in total, approximately a ten-fold increase in 2017.<sup>1</sup> This accounts for nearly 50% of deaths investigated by the Office of the Chief Medical Examiner (OCME) in Maryland.

Using OCME death records for the State of Maryland, a retrospective study was performed to examine drug-related deaths over a five-year period from 2013 to 2017. The OCME is a statewide medical examiner's system, and therefore has jurisdiction over the entire state with a total population of 6,052,177.<sup>2</sup> This study showed that there were 7,869 drug-related deaths from 2013 to 2017, which was a 60.2% increase from 928 in 2013 to 2,333 in 2017. Death rates were analyzed as fatalities per 100,000 population. There was a consistent rise in death rate across the state of Maryland; however, Baltimore showed the most significant rise, from 43.8/100,000 in 2013 to 127.2/100,000 in 2017.

Total drug deaths steadily increased from 2013 to 2015 (31.2%), with a sharp rise between the years 2015 to 2017 (approximately 73%). The White and African American ratio was 2.4:1. The male and female ratio was 2.3:1.

The deaths were arranged by age into ten-year intervals. Data showed that the age of the decedents increased, with the highest percentage (76.7%) in the age group 61–70 years old. This may indicate a new trend in drug abuse deaths for the elderly. The age group of 31–40 years old also increased significantly by 65%.

Fig. 1 shows the trend of all the drug abuse deaths in the past five years. The data revealed that the sharp rise in drug abuse deaths was caused by fentanyl/fentanyl analogue intoxication. In 2013, there were only 60 deaths due to fentanyl intoxication, while in 2017, there were 1,610 fentanyl-related deaths, nearly a 27-times increase. Of the 1,610 fentanyl-related deaths, 820 (50.1%) were caused by mixed fentanyl with cocaine and/or heroin/methadone. The OCME also witnessed a significant increase in deaths due to multiple fentanyl analogues, including acetylfentanyl, butyrylfentanyl, despropionyl fentanyl, furanyl fentanyl, 4-fluoroisobutyryl fentanyl, arylfentanyl, tetrahydrofuran fentanyl, methoxyacetyl fentanyl, and cyclopropyl fentanyl, as well as carfentanil.

Fentanyl/fentanyl analogues and fentanyl mixed with heroin and/or cocaine abuse has become an epidemic in Maryland. This study recommends that more aggressive regulatory, educational, and treatment measures are necessary to address the tremendous increase in fatal drug abuse deaths, especially fentanyl-related deaths.

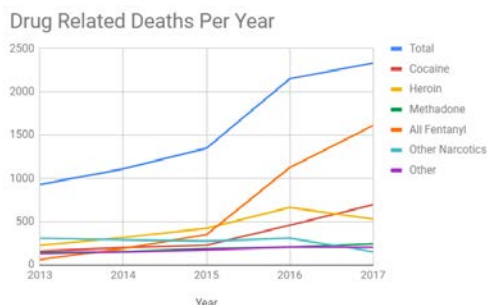


Figure 1

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### Drug Death, Overdose, Fentanyl

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### H175 Fatal Rodenticide Poisoning in Association With Synthetic Cannabinoid Use—Is It Industrial Contamination?

Rochelle A. Simon, MD\*, Baltimore, MD; David R. Fowler, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223; John Stash, DO, Office of the Chief Medical Examiner, Baltimore, MD 21223

**Learning Overview:** The goal of this presentation is to illustrate the clinicopathologic features of vitamin K-dependent antagonist coagulopathy associated with the use of tainted synthetic cannabinoids and explore a hypothesis of how the cannabinoids came to be contaminated with rodenticide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by alerting investigators and pathologists to the potentially fatal coagulopathy that may occur with the use of synthetic cannabinoids tainted with brodifacoum, exploring a hypothesis of contamination, and discussing prospective testing to evaluate this hypothesis.

Synthetic cannabinoids have been used recreationally to mimic the psychotropic effects of marijuana, and there has been a relatively recent increase in the number of associated fatalities. These compounds are reportedly consumed as an alternative to cannabis because of accessibility, enhanced effects, and relative undetectability, among other reasons. Synthetic cannabinoids are more variable in content, potency, and function when compared to naturally occurring cannabis. Recent outbreaks of life-threatening vitamin K-dependent antagonist coagulopathy in association with synthetic cannabinoid use have been reported, affecting the states of Illinois, Maryland, Indiana, Missouri, and Wisconsin.<sup>1</sup>

This case involves a 33-year-old male synthetic cannabinoid user who presented to emergency medical services with complaints of hematuria and penile bleeding. The patient went into cardiac arrest before reaching a hospital and resuscitative efforts were ultimately unsuccessful. Postmortem examination identified numerous contusions on the extremities and torso, while analysis of hospital blood showed low hemoglobin (4.0g/dL) and hematocrit (13.1%) with elevated Prothrombin Time (PT of 316.3secs) and International Normalized Ratio (INR of 26.6). Internal examination revealed soft tissue hemorrhage around multiple organs, generalized organ pallor, and gastrointestinal hemorrhage. Toxicologic analysis performed on hospital blood revealed the presence of brodifacoum, a second-generation anticoagulant rodenticide. These clinicopathologic findings are similar to those recently reported in fatal and non-fatal incidents occurring across regions of the United States.

Investigations to determine how rodenticide may have been introduced into synthetic cannabinoids in these life-threatening cases are ongoing. Speculative reports of possible intentional lacing exist in the literature; it has been hypothesized that since both rodenticides and synthetic cannabinoids are metabolized in the liver, saturation of liver enzymes with rodenticide may potentiate and prolong the desired psychotropic effects of the drug.<sup>2</sup> A more worrisome concern is that synthetic cannabinoids are being contaminated intentionally with brodifacoum to cause death. However, a more likely hypothesis is that synthetic cannabinoids may be contaminated at the manufacturing level, during production or storage.

Due to its long half-life and consequent ability to kill after a single exposure, brodifacoum has been banned as a readily accessible, small-quantity, consumer product in the United States.<sup>3</sup> Brodifacoum, and others in this class (such as bromadiolone), are only available for commercial use. In contrast, in China, where most synthetic cannabinoids are manufactured, these rodenticides are widely available. In fact, reports have implicated brodifacoum second only to bromadiolone in the incidence of rodenticide poisoning in this region.<sup>4</sup>

The relative prevalence of accidental brodifacoum poisoning and accessibility of brodifacoum in China raise the possibility of industrial contamination of synthetic cannabinoids outside of the United States, which may produce a fatal coagulopathy. Toxicologic evaluation for brodifacoum in all cases of synthetic cannabinoid use may help to determine the extent of contamination, as low levels may be present in non-fatal or non-life-threatening cases, in addition to fatal cases of acute synthetic cannabinoid intoxication. It is recommended that all autopsy cases which test positive for synthetic cannabinoids also be screened for brodifacoum and other second-generation vitamin K-dependent anticoagulants to evaluate the extent of contamination.

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#### Brodifacoum, Cannabinoids, Coagulopathy



### H176 An Excipient-Induced Oxycodone Fatality

*Deanna A. Oleske, MD, District 23 Medical Examiner's Office, St. Augustine, FL 32095; Drake Ryan Thrasher, BS\*, Birmingham, AL 35205; Jeff Walterscheid, PhD, Quality Forensic Toxicology, San Antonio, TX*

**Learning Overview:** After attending this presentation, attendees will be able to differentiate death due to injecting extended-release tablet formulations instead of relying specifically on low concentrations of oxycodone in postmortem blood.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of oxycodone in fatal toxicity and challenging assumptions of lethal ranges when considering the adverse vascular effects of parenteral administration of an extended-release tablet.

Opiates such as oxycodone are among a class of semi-synthetic analgesic narcotics indicated for relief of postsurgical pain. Opiate toxicity manifests through its sympatholytic properties of decreased cardiac output and respiratory depression, along with observable side effects such as urinary retention and miosis. Extended-release preparations of oxycodone are often formulated with polyethylene oxide to reduce dosing frequency and curb abuse by preventing rapid absorption. When used outside the parameters of intended delivery, such as intravenous injection, the user is abusing the drug to achieve a larger dose in a shorter time.

However, intravenous delivery creates a condition in which the extended-release excipients can cause fatality without the accompanying signs of opiate intoxication and result in relatively low postmortem concentrations of oxycodone. Patients reporting to emergency care will paradoxically suffer from shortness of breath, fever, and diarrhea. In these cases, Thrombotic Thrombocytopenic Purpura (TTP) -like illnesses later determined to be thrombotic microangiopathy have been diagnosed in patients who crush and inject tamper-resistant opiates (e.g., oxycodone and tapentadol). This is likely due to vascular inflammatory reactions and hemolysis from particulates in the suspension. Although thrombotic microangiopathy and TTP-like illnesses have been reported in the literature from intravenous injection of tamper-resistant opiates, none to date have been described in the forensic literature with appropriate quantitative toxicologic analysis.

Presented here is a case in which the decedent created and injected a formulation consisting of crushed oxycodone and tapentadol tablets mixed into a solution containing promethazine and meperidine. The decedent was found approximately eight hours after sending a voice-to-text message, with slurred slow speech, stating his last wishes. A tourniquet was in place around the upper arm with two injection marks in the antecubital fossa. The decedent was clutching a 50-milliliter syringe with a large bored needle that contained a clear, brown-tinged, viscous fluid. Autopsy findings were consistent with a typical opiate-associated fatality (pulmonary edema, urinary retention). Review of the prescription drug database showed the decedent had chronic opiate dependence, currently taking oxycodone, with a remote prescription for tapentadol. No prescription bottles were found on scene.

The toxicology showed a number of substances, including ethanol, diazepam, oxycodone, meperidine, methocarbamol, tapentadol, and promethazine. However, all were within moderate concentrations and inconsistent with fatal toxicity. Microscopic examination showed endothelial swelling of the arteries in the kidney and fragmented red blood cells (schistocytes) consistent with thrombotic microangiopathy.

This presentation shows how, under these circumstances, the mechanism of death should be carefully elucidated in deaths resulting from intravenous drug abuse of tamper-resistant substances with less reliance and emphasis on reference ranges of the measured drug concentrations. In conclusion, this study describes a death resulting from injecting crushed tamper-resistant, extended-release oxycodone and tapentadol tablets.

#### Oxycodone, Extended-Release, Intravenous



### H177 A Medical Examiner's Office Review of Mechanisms of Injury in Crush-Related Deaths

Catherine Rood, BS\*, Syracuse University, Syracuse, NY 13244; Robert J. Bready, MS, Dutchess County Medical Examiner's Office, Poughkeepsie, NY 12601; Dennis J. Chute, MD\*, Dutchess County Medical Examiner's Office, Poughkeepsie, NY 12601

**Learning Overview:** The goal of this presentation is to show attendees how rare traumatic asphyxia is reported and what other mechanisms of injury are named in crush-related deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by indicating what mechanisms of injury pertain to crush-related deaths.

Entrapment from cars, trees, and other work-related machinery producing crushing injuries and traumatic asphyxiation are rare.<sup>1,2</sup> Traumatic asphyxia, also known as crush or compression asphyxia, is caused by external pressure on one's body that compresses the chest and abdomen, preventing respiration, the uptake of oxygen, and removal of carbon dioxide.<sup>3</sup>

Typical pathological findings in such asphyxia deaths include petechiae of the face, neck, and upper chest, congestion, cyanosis, subconjunctival hemorrhaging, edema, and abrasions. In the blunt force trauma deaths, rib, sternum, and spine fractures, lung contusions, transection of the aorta, and lacerations to organs may be found. At autopsy, the pathologist may find evidence of asphyxia and blunt force trauma. This may cause discrepancies as to how the cause is to be certified. In a retrospective study of cases brought into the Dutchess County Medical Examiner's Office over a 14-year period, 17 out of 11,260 (0.15%) cases were identified in which an individual was crushed by a heavy object. This study was specifically interested in what were the mechanisms of death. In this review, this study found five cases (29%) were certified as due solely to crush/compressional asphyxia, nine cases (53%) to blunt force trauma, and three deaths (18%) attributed to a combination of the traumatic asphyxia and blunt trauma. Three examples are presented below.

**Case 1:** A 19-year-old male was found pinned under a vehicle after a scissor jack collapsed. The deceased was last seen alive by his grandmother 25 minutes prior to finding him. Autopsy findings noted abrasions of the upper abdomen, petechiae on the face, neck, and upper chest, and mild brain edema. The medical examiner ruled his death an accident by compressional asphyxia.

**Case 2:** A 57-year-old male was crushed by a cinder block-making machine on the job. The deceased was working on the machine when it trapped him between a metal brace and a metal plate holder. A coworker witnessed the event and immediately hit the emergency break to reverse the machine. Autopsy findings noted patterned abraded contusions of the chest and back, fractures of the sternum and multiple ribs, bilateral hemothoraces, bilateral lung contusions, and transection of the aorta with hemorrhaging. The medical examiner ruled his death an accident by crushing injury to the chest with thoracic fractures and injuries to the lungs and aorta.

**Case 3:** A 58-year-old male was pinned by a steel beam at a construction site. The deceased was operating an excavator while demolishing a building when a roof beam fell from the collapsing building and entered the cab of the excavator. A coworker filmed the event as it proceeded. Autopsy findings noted petechiae of the eyelids, conjunctivae, face, neck, and upper torso, and depressed right chest wall associated with right rib cage fractures. The medical examiner ruled his death an accident due to a combination of blunt impact injuries of the chest and compressional asphyxia.

**Conclusion:** Traumatic asphyxia can occur under varied circumstances. In these cases, scene investigation may help the forensic pathologist reconstruct the sequences of events, provide insights as to why the decedent was unable to escape, and how quickly the circumstances transpired. An estimate of the period of survival may be assessed by witness accounts and the injuries discovered at autopsy. In addition to hallmark features that help medical examiners determine mechanism of death, one should consider whether a reflex closure of the glottis occurred, as has been speculated by Byard et al. (i.e., when there is torso compression, the glottis closes from reflex deep inspiration).<sup>4</sup> This reflex produces an increase in central venous pressure, which results in suffusion of the head.<sup>5</sup> Such reconstructions and examinations into mechanisms of death have practical applications toward preventing work-related accidents as well as for future legal proceedings. For example, forensic pathologists may be called to opine in civil litigations as to whether there was conscious pain and suffering at the time of the event. In review of the cases, this study found a significant number of these deaths involved blunt force trauma and the minority purely traumatic asphyxiation.

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#### Traumatic Asphyxia, Blunt Force Trauma, Crushing Deaths



## H178 Blunt Force Decapitation in West Tennessee: A Ten-Year Review (2008–2018)

William Sago, MD\*, Memphis, TN 38105; Paul V. Benson, MD, University of Tennessee Health Science Center/Shelby County Medical Examiner, Memphis, TN 38105

**Learning Overview:** After attending this presentation, attendees will understand the accidental and suicidal blunt force decapitation injury case characteristics, postmortem findings, and reports of circumstances commonly associated with these cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting a rare type of death by reviewing literature and multiple cases from West Tennessee.

Decapitation due to blunt force injuries is a rarely seen cause of death in most death investigation jurisdictions. Although decapitation due to blunt force injuries is a rare cause of death in civilian populations, it is reported to have occurred in the United States and various other countries.<sup>1-4</sup> Accident and suicide accounted for all the manners of death in the cases reviewed. Blunt force decapitation suicides due to train-induced trauma are reported.<sup>2</sup> Rare and unusual cases have been reported of suicides with self-decapitation by hanging and as motor vehicle-assisted decapitations.<sup>3</sup> Accidental decapitations due to traffic accidents and failure to use seat belts have also been reported.<sup>4</sup>

This study provides a ten-year review of decapitation due to blunt force injuries in cases referred to the West Tennessee Regional Forensic Center in Memphis, TN, including cases from 3 of the 13 counties served by the West Tennessee Regional Forensic Center including Shelby County, Fayette County, and Obion County. Methods included searching the West Tennessee Regional Forensic Center database to extract data based on coding of the cause of death. Results showed seven deaths resulting from blunt force decapitation between 2008 and 2018 in West Tennessee with the most recent case occurring in 2015. The yearly distribution of these seven cases within the past ten years ranged from one death in 2008, one death in 2009, one death in 2010, no deaths in 2011, no deaths in 2012, two deaths in 2013, one death in 2014, one death in 2015, no deaths in 2016, no deaths in 2017, and no deaths in 2018.

All the decapitation deaths due to blunt force trauma studied were White males. The ages of the victims ranged from 14 to 69 years old. Three of the deaths were reported as suicidal manner of death. One suicidal death involved blunt force injuries due to a train. The remaining two suicidal deaths involved motor vehicle-assisted decapitation with a ligature tied to a stationary object. Of these two motor vehicle-assisted decapitation suicidal deaths, one decedent was restrained by a seat belt and the other was not. One suicidal decapitation by train and one motor vehicle assisted decapitation case included a suicide note. One motor vehicle-assisted decapitation suicide did not have a suicide note. The remaining four deaths were reported as accidental manner of Death. Two of the accidental cases involved a motor vehicle crash, one accidental case involved a pedestrian hit by motor vehicle, and one accidental case was due to an aircraft crash. Review of autopsy reports, autopsy photos, and scene photos showed one case coded as decapitation was a partial decapitation and two cases coded as decapitation were decapitation from the mandible superiorly. The aircraft crash case involved fragmentation of the decedent with decapitation.

This retrospective study provides a review of blunt force decapitations reported to the West Tennessee Regional Forensic Center from 2008 to 2018. Although death due to blunt force decapitation is rare, the seven cases reported at the West Tennessee Regional Forensic Center show some similarities to other cases reported throughout the world.

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### Decapitation, Blunt Force, Suicide

## H179 Accidental Residential Fire-Related Fatalities: A Seven-Year Study in a Forensic Autopsy Population (2010–2017)

Allison C. Klein, BA\*, University of Maryland Baltimore, Graduate School, Baltimore, MD 21201; Kelsey Mason, BS\*, University of Maryland Baltimore, Baltimore, MD 21201; David R. Fowler, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223; Mary G. Ripple, MD, Baltimore, MD 21223; Ling Li, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223

**Learning Overview:** After attending this presentation, attendees will learn the groups of people at risk for accidental fire-related fatalities, the conditions which predispose residences to fire, and the epidemiological implications for studying fire deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing its understanding of the risk factors and potential fire prevention measurements in fire-related deaths. It is also important for forensic investigators to document whether a fire or smoke detector was present or functional in any fire-related death investigation.

The majority of deaths from fire are accidental residential events, caused by smoking, electrical malfunctions, improper usage of faulty heating, and clothing igniting on fire from cooking accidents.<sup>1</sup>

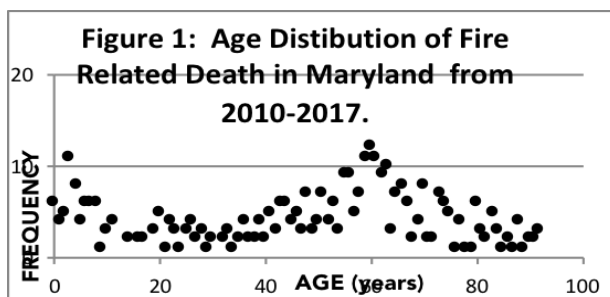
Using death records obtained from the Office of the Chief Medical Examiner (OCME) for the state of Maryland, a retrospective study was conducted on fire fatalities over a seven-year period (2017–2010). The OCME is responsible for conducting death investigations and certifying the cause and manner of unnatural and unexplained deaths, including homicide, suicide, unintentional injuries, deaths that are unattended, or sudden unexpected deaths in the State of Maryland. The data collected at OCME provides an invaluable source of data for identifying statewide epidemiological trends, which affect public health and, therefore, aid in the identification of at-risk groups.

This study revealed that OCME investigated 378 cases of fire fatalities over the ten-year period. Of those, 309 (81%) deaths were due to accidental fire, 19 (5%) deaths were homicides, and 18 (4.7%) cases were suicides. There were 32 (8.5%) deaths with manner of death undetermined. The male-to-female ratio was 2:1.4. The ages of the victims range from 9 months to 92 years, with an average age of 46.95 years. The age groups with the highest frequency of deaths were the elderly (>60 years old) and juveniles (<18 years old) (Fig 1). Many elderly individuals live alone and may not maintain electrical appliances as they should.<sup>2</sup> The isolation of elderly victims and the potential inability to ambulate quickly, or at all, constitutes two main reasons this population is at risk of fire death. Infants and juveniles must rely on assistance from others to escape fires unharmed. A lack of sufficient escape route planning may also contribute to the increased number of juvenile victims.

Of the 309 accidental residential fire deaths, 56 (18%) were caused by smoking. Six victims were smoking cigarettes while receiving oxygen therapy for cardiovascular and/or respiratory diseases. In approximately 8% of cases, the source of fire was attributed to electrical malfunction or heating sources. In addition, cooking implements left unattended were the source of 3% of deaths in the home.

One of the objectives of this study was to identify the presence and function of fire or smoke detectors. However, the data concerning fire alarm systems was often not recorded or unknown in the death investigation reports. It is important to document whether a fire or smoke detector was present or functional in any fire-related death investigation. Smoke alarms provide an early warning of a fire, giving people additional time to escape, and working smoke alarms reduce the risk of fire-related deaths.

Residential fire prevention efforts should target home safety education focused on the elderly and children to maximize survival for these at-risk groups. Education regarding the combustibility of smoking materials and the hazards of smoking on oxygen therapy must also be directed at those at risk and their caretakers. The development of easily maintained and technologically advanced fire and smoke monitoring systems could also aid in the prevention of fire death and aid in future epidemiological research.



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### Fire Death, Elderly Victims, Accident



### H180 Trousseau's Syndrome in Forensic Medicine

Henry J. Carson, MD\*, Cedar Rapids, IA 52411

**Learning Overview:** After attending this presentation, attendees will be able to: (1) recognize migratory thrombophlebitis associated with malignancy in forensic cases, (2) apply principles of thromboembolic processes to cause and manner of death, and (3) provide usable information to survivors about unexpected medical conditions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping improve competence to correlate thromboembolic phenomena with carcinoma and improve patient outcomes by providing detailed information for reports that answers more questions and provides a valuable mechanism of death.

In 1865, Armand Trousseau described a syndrome of migratory thrombophlebitis as a complication of carcinoma. Understanding this syndrome may provide insight to the underlying mechanism of death in certain cases. Two patients were encountered who died from cardiovascular disease that was proximally related to complications of occult gallbladder carcinoma and the thromboembolic complications of Trousseau's syndrome.

The first subject was an 83-year-old White woman who was admitted to the hospital for congestive heart failure. She died shortly after admission. Autopsy demonstrated evidence of heart failure with thrombosis of the left anterior descending coronary artery, but also an adenocarcinoma of the gallbladder. It was concluded that the patient died from complications of an acute thrombosis of the left anterior coronary artery that could be related to the thromboembolic phenomenon of Trousseau's syndrome.

The second subject was a 76-year-old man who had undergone cardiac bypass surgery one-week antemortem. He had returned home and died after a short walk. At autopsy, numerous formed thromboemboli were recovered from the pulmonary arteries. Incidentally, the gallbladder had a well-differentiated adenocarcinoma. It was concluded that the patient died from pulmonary thromboembolism, which could have been related to thromboembolic phenomena of Trousseau's syndrome.

In the United States, gallbladder carcinoma has an incidence of 1.13 cases per 100,000 population. Women are three times more likely to develop gallbladder carcinoma than men. The most consistent anatomical correlation with gallbladder carcinoma is cholelithiasis. The development of Trousseau's syndrome, which can lead to thromboembolism, can occur in 1%–11 % of cancer patients. Pancreatic cancer is most highly correlated with Trousseau's syndrome and Pulmonary Embolism (PE), but other malignancies are associated with this syndrome. Trousseau's syndrome can be seen as a chronic form of disseminated intravascular coagulation or a prothrombotic state, in which cancer leads to hypercoagulability by various mechanisms. The role of thromboembolism in these patients establishes a mechanism of death by forming blood clots that obstructed critical blood vessels.

While medical data sufficient to affirm a cause of death based on clinical circumstances were available in the present cases, autopsies provided more information about the mechanism of death in these patients, and provided comfort to the survivors in both families that the outcomes were more bearable, especially in light of the autopsy findings, in which an elusive, serious cancer was found, which would have caused considerable morbidity and likely eventual fatality if the family member had not died beforehand from competing causes.

**Forensic Science, Thromboembolism, Gallbladder Neoplasms**



## H181 Exercise-Associated Hyponatremia: An Update

Paul Uribe, MD\*, Martin Army Community Hospital, Fort Benning, GA 31905

**Learning Overview:** After attending this presentation, attendees will better understand the critical nature of recognizing Exercise-Associated Hyponatremia (EAH) as a potentially fatal complication of overhydration and extreme exertional exercise that requires rapid diagnosis and treatment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) introducing attendees to this subset of acute hyponatremia that occurs exclusively in elite athletic populations; and (2) explaining the underlying physiology, the importance of rapid diagnosis and treatment, and the far-reaching implications of this condition for diagnostic and treatment algorithms developed for military training exercises.

EAH is a preventable condition associated with overconsumption of hypotonic fluids in a setting of extreme physical exertion that exceeds the renal capability to diurese.<sup>1</sup> EAH is seen in elite athletic populations, such as marathon runners, triathletes, and military training populations.<sup>2</sup> Long-held misconceptions regarding how much fluid intake is required during exercise exacerbates the problem of EAH.<sup>3</sup> The treatment of severe hyponatremia has traditionally focused on correcting the sodium deficit slowly to prevent the potentially lethal complication of central pontine myelinolysis. This traditional approach for the treatment of chronic hyponatremia does not apply to the current model of EAH in this population of elite athletes. The drop of serum sodium is due to water overconsumption during extreme exercise, can occur rapidly, and must also be corrected rapidly to avoid the fatal complication of irreversible cerebral edema, also termed Exercise-Associated Hyponatremic Encephalopathy (EAHE). Once recognized, the sodium deficiency must be corrected with 3% hypertonic saline as soon as possible. Rapid correction of the sodium deficits in acute exertional hyponatremia does not result in the classic complication of central pontine myelinolysis and avoids the complication of EAHE.<sup>4</sup> If treated per the traditional model in which the sodium deficit is corrected gradually, the use of 0.9% saline or other isotonic solutions in these patients can worsen volume overload, This leads to potentially fatal complications, such as pulmonary edema and EAHE.

The underlying physiology of EAH is hallmarked by the sodium-conserving actions of anti-diuretic hormone. These patients develop Syndrome Of Inappropriate Antidiuretic Hormone (SIADH) in which vasopressin is paradoxically released, likely as a sodium conservation measure.<sup>5</sup> This increase in vasopressin allows the renal tubules to conserve salt and also retain water, which prevents the usual diuresis from fluid resuscitation or continued overhydration. This inability to off-load excess fluid greatly predisposes these patients to pulmonary edema and ultimately irreversible cerebral edema.

This presentation reviews three fatal cases of EAH, examines the underlying physiology behind EAH, and reviews the diagnosis and treatment algorithms currently in development by the United States Army.<sup>6</sup>

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### Hyponatremia, Exercise, Electrolytes





### H182 Sudden Unexpected Death in Pemphigus Vulgaris: An Autopsy Report

Asit Kumar Sikary, MD\*, ESIC Medical College & Hospital, Faridabad, Haryana 121001, INDIA

**Learning Overview:** The goal of this presentation is to discuss sudden death in a case of pemphigus vulgaris. The fatal event was precipitated by the steroid used for the treatment of a skin disease.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by suggesting that the forensic pathologist should be aware of the medication history of the deceased and any complications of the medications, even when the dose is well regulated.

Sudden deaths form a significant proportion of autopsies globally. The incidence of sudden deaths varies globally owing to the variations in the prevalence of various diseases in different countries and environmental and genetic factors. The incidence of sudden death has been reported to vary from 1.8% to 31% in various studies worldwide. While most of the sudden deaths are attributed to cardiac and respiratory causes, dermatological conditions are rarely implicated in sudden deaths.

As per the World Health Organization (WHO), the death rate due to skin diseases in India is about 1.63 per lac population. Most common fatal skin diseases include Harlequin ichthyosis, epidermolysis bullosa, Ehlers-Danlos Syndrome, methicillin-resistant *Staphylococcus aureus*, toxic epidermal necrolysis, basal cell carcinoma, squamous cell carcinoma, scleroderma, leishmaniasis, and pemphigus, among others. The literature on complications of steroid therapy in pemphigus patients is limited.

A 57-year-old non-alcoholic, non-hypertensive, and non-diabetic man was diagnosed with severe pemphigus vulgaris 14 weeks prior to his death and was under systemic steroid therapy (oral prednisone 80mg/day) since then. His skin lesions were improving; however, his blood pressure recorded during the last follow up, one week before death, was 150/100mm Hg and pulse was 100/min. During the 14 weeks of treatment, the patient had gained 10kg weight. On the fateful day, the deceased collapsed while taking a bath in the morning and was brought to the emergency department of a hospital where he was declared dead on arrival.

At postmortem examination, pemphigus lesions were present over the trunk and thighs evenly in the form of collapsed blisters, erosions, and crusting. Inflammatory and healing changes were seen in some of the lesions over the thighs. Histopathological examination of skin lesions confirmed pemphigus vulgaris with intraepidermal separation of keratinocytes and “rounding off” at the suprabasal layer. The lungs weighed 580gm each, and on histopathology, showed severe interstitial lung disease with capillary congestion, and hemosiderin laden heart failure cells with old hemorrhages. The liver weighed 2,017gm and showed micro vesicular steatosis with focal vascular dilatation and congestion on microscopy. The spleen showed congestion of red pulp with depletion of white pulp and medial hypertrophy of blood vessels on microscopic examination. The heart was enlarged and weighed 518gm without any valvular or aortic outflow obstruction. Hypertrophy of cardiac myocytes was observed on histopathology. Renal vasculatures showed medial hypertrophy with intimal thickening and mild peritubular capillary congestion on histopathology. The vitreous glucose level was 105gm/dl.

These findings suggest that the deceased was suffering from chronic systemic hypertension and chronic cardiac failure, which was potentially aggravated by the steroid treatment, causing sudden collapse.

#### Autoimmune Disease, Hypertensive Changes, Steroid Therapy



### H183 Sudden Death Caused by Bilateral Diaphragmatic Eventration in Myotonic Dystrophy Type 1

Sulin Wu, PhD\*, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49007; Joseph A. Prahlow, MD, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49007

**Learning Overview:** After attending this presentation, attendees will understand the pathophysiology and genetic basis of myotonic dystrophy, a multisystem inherited disorder that represents the most common muscular dystrophy observed in adults.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting a case of sudden death related to respiratory compromise caused by bilateral diaphragmatic eventration and associated restrictive lung disease in a woman with a known clinical diagnosis of Myotonic Dystrophy Type 1 (MD1).

A 48-year-old woman with a known clinical diagnosis of MD1 was found unresponsive in her home. She was pronounced dead at the scene. A clinical diagnosis of MD1 had been rendered at the age of 13 years, although no genetic/molecular testing had ever been performed. At 39 years of age, she was diagnosed with chronic obstructive pulmonary disease and restrictive lung disease.

A medicolegal autopsy was performed, which disclosed a mildly obese (BMI=29.4) adult female. On internal examination, the lungs appeared somewhat hypoplastic, with a combined weight of 470 grams. The diaphragm was intact, but was markedly elevated bilaterally, with associated decrease in pleural cavity volume. Much of the diaphragm was parchment-thin and nearly translucent. Additional gross findings at autopsy included changes consistent with hypertensive and atherosclerotic cardiovascular disease. Microscopic examination of the diaphragm showed deformation and degeneration of skeletal muscle, with fat and fibrous tissue replacement and residual muscle fibers containing numerous central nuclei and rare cytoplasmic vacuolization. The cause of death was ruled respiratory compromise due to restrictive lung disease due to bilateral diaphragmatic eventration due to MD1, with underlying hypertensive and atherosclerotic cardiovascular disease. The manner of death was natural.

Myotonic dystrophy is the most common muscular dystrophy observed in adults.<sup>1,2</sup> The condition is a multisystem disorder, characterized by muscle weakness, myotonia, cardiac conduction abnormalities, and various other manifestations, with cardiac and respiratory issues being responsible for most deaths.<sup>3-5</sup> MD1 is the most common form of the disorder, resulting from an autosomal dominant inheritance of a triplet-repeat disorder.<sup>3,4</sup> The presented case is a bit unusual in that she was diagnosed with the juvenile form of MD1 but experienced only limited clinical manifestations for most of her adult life. Respiratory involvement in the disorder is a complex phenomenon, related to variable involvement of chest wall muscles and the diaphragm, with associated apnea and chronic hypoxemia; restrictive lung disease in MD1 patients is a recognized indicator of cardiac events and risk of death.<sup>6,7</sup> A recently published case of MD1-related death emphasizes the fact that fat-replacement of muscle can contribute to death.<sup>8</sup> In the present case, the marked diaphragmatic eventration, with associated pulmonary compromise, represents another unusual manifestation of MD1. Clinicians and pathologists should remain aware of this rare presentation of MD1.

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#### Myotonic Dystrophy, Diaphragmatic Eventration, Death



### H184 Mutations of Plakophilin-2 (PKP2) in Sudden Unexplained Death (SUD)

*Junyi Lin, Shanghai, CHINA; Bi Xiao, Shanghai, CHINA; Ziqin Zhao, MS, Shanghai, CHINA; Sisi Cheng, Shanghai, CHINA; Yiwen Shen, Shanghai, CHINA; Mingchang Zhang\*, Department of Forensic Medicine Fudan University, Shanghai 200032, CHINA*

**Learning Overview:** After attending this presentation, attendees will know PKP2 mutations may occur in cases of SUD, specifically in patients without evidence of fibrofatty change, and that PKP2 protein defect in itself is arrhythmogenic.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing attendees to the results of DNA sequencing of PKP2 extracted from postmortem heart tissues of 25 Western patients dying from SUD and 25 Chinese Han patients dying from SUD.

SUD remains a puzzle in forensic medicine. PKP2 has been linked to Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), which may cause life-threatening ventricular arrhythmias and sudden death. Fatal arrhythmias resulting in sudden death also occur in the absence of morphologic cardiac abnormalities at autopsy and have been linked to ion channel mutations in a subset of cases, but so far not to PKP2. This study sequenced all 14 exons of PKP2 in DNA extracted from the postmortem heart tissues of 25 Western patients and 25 Chinese Han patients dying from SUD. The primers were designed using the Primer Express 3.0 software. Direct sequencing for both sense and antisense strands was performed with a BigDye Terminator DNA sequencing kit on a 3130 xl Genetic Analyzer. Mutation damage prediction was made using Mutation Taster and Polyphen. In 6 of the 25 cases of Western SUD samples, 6 PKP2 mutations (p.F339S, P665S, p.P665S, p.Y217TfsX45, p.E540, and p.S615T) were identified, 3 of which were probably pathogenic according to Mutation Taster and Polyphen. In 2 of 25 cases of Chinese Han SUD samples, 2 PKP2 mutations (p.R691Q, p.L366P) were identified, 1 of which was probably pathogenic according to Mutation Taster and Polyphen. The present study also confirms that the PKP2 mutation (p.Y217TfsX45) which was found in a patient dying of SUD could reduce the expression of PKP2 and could also promote the autophagy to decrease the expression of Cx43.

Data collected in this study suggest a link between the PKP2 mutations and SUD cases in both Western and Chinese Han population. However, PKP2 mutations are more frequent in Western SUD cases than in Chinese Han population cases and have complications in that medical examiners who perform molecular genetic screening in cases of SUD need to be aware that PKP2 mutations may also be able to cause fatal arrhythmias even in patients with a morphological normal heart.

#### **Plakophilin-2, Desmosomal Mutation, Sudden Unexplained Death**

### H185 The Use of High-Mobility Group Protein (HMGB1) to Determine Time Since Death: An Autopsy-Based Study

Puneet Setia, MD\*, All India Institute of Medical Science, Jodhpur, Rajasthan, INDIA; Rutwik D. Shedge, MSc, All India Institute of Medical Sciences, Jodhpur 342005, INDIA; Anupama Modi, MSc, All India Institute of Medical Sciences, Jodhpur 342005, INDIA; Purvi Purohit, PhD, All India Institute of Medical Sciences, Jodhpur 342005, INDIA; Praveen Sharma, All India Institute of Medical Sciences, Jodhpur 342005, INDIA

**Learning Overview:** After attending this presentation, attendees will be informed regarding the importance of Enzyme Linked Immune-Sorbent Assay (ELISA) utility in the evaluation of time since death. The goal of this presentation is to update attendees regarding the use of HMGB1 as an easy, cheap, and reliable marker to determine time since death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a new method to determine time since death, which can be utilized even at the grass root level.

Determining time since death is one of the most important aspects of forensic work. Since time immemorial, time since death has been estimated using various techniques. The earlier standards included checking livor mortis, hypostasis, rigor mortis, signs of decomposition, forensic entomology, etc.<sup>1</sup> These standards are of a subjective nature and depend upon non-scientific aids such as gross observation for their recording. Since these were never accurate, the quest for better methods led to the development of postmortem biochemistry, especially in the vitreous humor.<sup>2,3</sup> The major shortcoming of this method was that the results were applicable only for a short postmortem interval, merely a few hours.<sup>4,5</sup>

A group of non-histone nuclear proteins with high electrophoretic mobility were discovered in 1973 and were termed High Mobility Group (HMG) proteins. These include three superfamilies, HMGB, HMGN, and HMGA. Out of these, HMGB1 is the most abundant as well as the most well-studied protein. HMGB1 is an important protein that plays a critical role as a DNA chaperone, chromosome guardian, autophagy sustainer, and protector from apoptotic cell death. HMGB1 is released by eukaryotic cells upon necrosis. This property has been made use of in estimating Postmortem Interval (PMI) in animal models using Wistar rats.<sup>6,7</sup>

This presentation is part of an ongoing study on the determination of time since death using serial estimation of HMGB1 in blood, liver, spleen, and brain tissues harvested during routine medicolegal autopsy. The selection criteria for the cadavers is in-hospital death undergoing medicolegal autopsies, and those who do not have any condition that increases tissue necrosis/cell death. The blood and tissue samples are removed at the time of the autopsy and further subjected to extraction of serum and tissue supernatant (in phosphate-buffered saline) at regular time intervals of zero, three, six, and nine hours. The levels of HMGB1 in serum and tissue supernatant are being assessed using a commercially available ELISA kit. The initial results have shown positive correlation between the HMGB1 levels and time since death. The results of various tissues, such as liver, brain, spleen, and blood, and their correlation will be presented. The use of ELISA as the method for analysis makes it a cheap, rapid, and easily available procedure that can be utilized even in the busy mortuaries of developing countries as well as in the peripheral centers where facilities for advanced research and analysis are not available.

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#### High-Mobility Group Protein (HMGB1), Time Since Death, Enzyme-Linked Immuno-Sorbent Assay



### H186 Fatal Angioedema Due to a Delayed Hypersensitivity Reaction Associated With Hair Dye and a Temporary Tattoo

Ami L. Jackson, DO\*, Winston-Salem, NC 27104; Jerri McLemore, MD, Wake Forest School of Medicine, Winston-Salem, NC 27157

**Learning Overview:** The goal of this presentation is to increase awareness of a possible hypersensitivity reaction at autopsy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by enforcing the need for thorough investigation of the circumstances surrounding death, including friend and family interviews.

A young woman was found dead on her bedroom floor after complaining of a sore throat the previous evening. Two days prior, the decedent sat through a five-hour hair salon appointment to dye her hair a darker color. The decedent had dyed/bleached her hair a lighter color in the past, but this most recent hair treatment consisted of a dark color that she had not used before. She had complained of the fumes being too strong while her hair was being treated. Discussion with family members revealed no other changes in her daily routine, no history of recent exposures to new foods or chemicals, and no history of recent insect or other animal bites or exposures. She had no known history of allergies or predisposition to allergic reactions. Further investigation revealed the decedent had purchased a temporary tattoo from a European website approximately ten months prior to death.

At autopsy, the decedent had swollen lips and marked edema of the soft tissues of the larynx, the epiglottis, and vocal folds, along with periorbital and conjunctival petechiae. Microscopic analysis of the edematous tissue revealed an exuberant band of lymphoplasmacytic inflammatory cells in the submucosa with less intense mononuclear inflammation within the deeper tissue. A stain for mast cell tryptase was positive for numerous mast cells throughout the tissue. No other anatomic cause of death was found.

Hypersensitivity reactions, mainly in the form of allergic contact dermatitis or erythema, have been linked to a number of cosmetic products, including hair dyes. A common ingredient of darker tints of hair dyes is Paraphenylenediamine (PPD), an ingredient that imparts a dark color and is present in dark dyes used for fabrics; hair coloring products; paints and lacquers; cosmetics, particularly eye shadow; and shoe polish to name only a few items. PPD is also commonly used as the dark pigment in temporary tattoos, especially henna tattoos, sold outside of the United States. PPD derivatives have been implicated in the development of cross reactions and the development of angioedema in susceptible persons.<sup>1</sup> Fatalities associated with PPD usually involve intentional ingestion of this product as a means of suicide, predominantly in India.<sup>2</sup> Review of the medical literature uncovered one case report similar to this case of a delayed hypersensitivity reaction with angioedema connected to the use of a temporary tattoo followed by use of hair dye containing PPD a year later; however, no death was associated with this prior reported case.<sup>3</sup>

This case demonstrates the need for recognition of a possible hypersensitivity reaction at autopsy and thorough investigation, including extensive interviews with friends and family by medical examiners/coroners and death investigators, to pinpoint possible immunologic triggers. Outside of the fields of dermatology and cosmetology, the potential for this type of hypersensitivity reaction is not well known.

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#### Paraphenylenediamine, Delayed Hypersensitivity Reaction, Death



### H187 Fatal Clostridial Necrotizing Fasciitis Resulting From Skin Popping in Heroin Abusers

Hannah Elysse Bielamowicz, MD\*, Travis County Medical Examiner's Office, Austin, TX 78724; Kendall V. Crowns, MD, Travis County Medical Examiner's Office, Austin, TX 78724; Keith Pinckard, MD, PhD, Travis County Medical Examiner, Austin, TX 78701; Michelle S. Montonera, MS, Travis County Medical Examiner's Office, Austin, TX 78724

**Learning Overview:** After attending this presentation, attendees will gain a basic understanding of the production techniques of Black Tar Heroin (BTH) and its potential for bacterial contamination, examine the clinical presentation and autopsy findings of three cases of clostridial necrotizing fasciitis, and evaluate the pathophysiology of and risk factors associated with Necrotizing Soft Tissue Infections (NSTIs) in Injecting Drug Users (IDU) of heroin.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating the relative predominance of clostridial species in necrotizing fasciitis associated with subcutaneous or intramuscular injection of BTH. The relative prevalence of spore-forming bacteria in IDU-related NSTIs has been reported, but accounts of postmortem examination and pathologic findings in the forensic literature are sparse.

BTH has been the predominant form of heroin in the United States west of the Mississippi River since the 1980s. The initial production of BTH from opium requires boiling at temperatures of 150°C in acidic conditions, likely destroying any clostridial spores that may be present. Given this fact, contamination with bacterial species or spores most likely occurs via the addition of adulterants or during transport. Of note, during preparation for injection by the user, dissolution of heroin in an acidic solvent and heating to near-boiling temperatures effectively kills most bacteria, but these methods are ineffective at neutralizing clostridial spores.

Three adult individuals with histories of Intravenous Drug Abuse (IVDA) each presented to the hospital with severe soft tissue infections of their extremities secondary to skin popping of BTH. The nidus of infection in two cases was the shoulder, while that of the third case was the right thigh. Each individual was given intravenous antibiotic therapy and underwent surgical debridement of their wounds. One individual arrested mid-procedure and expired; the other two individuals succumbed to hypotensive shock less than 24 hours after surgery. Antemortem blood and tissue cultures of two cases grew *Clostridium sordellii*; the third case grew two species of *Clostridium*, not *C. perfringens*, which were unable to be further classified. Autopsy findings of all cases included at least one abscess of the extremity with surrounding erythema. Histologically, the lesions exhibited edema, acute inflammation, and necrosis of the skin, soft tissue, and muscle. Death in each case was attributed to *Clostridium sordellii* sepsis or necrotizing fasciitis and sepsis.

The major risk factor associated with the development of soft tissue infections caused by anaerobic spore-forming bacteria is skin popping. This leads to inflammation and devitalization of tissue, while also circumventing the bloodborne immune system that may otherwise thwart infection. Simultaneous injection of heroin with a vasoconstrictor such as cocaine generates tissue ischemia. All these factors promote anaerobic conditions for spore germination and toxin production.

Necrotizing fasciitis due to BTH injection appears to be primarily a clostridial disease, whereas the more typical pathogens are *Staphylococcus aureus* and *Streptococcus pyogenes*. *Clostridium sordellii* is among the most commonly isolated, in addition to *C. perfringens* and *C. novyi*. *C. sordellii* produces two cytotoxins that cause severe soft tissue edema, often without gas or purulence, marked leukocytosis, and rapidly fatal hypotensive shock. Interestingly, it has been isolated from BTH samples in prior studies.

Clostridial infection should be considered in an individual with a history of chronic narcotism and evidence of intramuscular or subcutaneous injection of heroin, the main risk factor associated with this disease. It should be noted that the region of inflammation may be subtle yet still cause death by a toxin-mediated illness. Care must be taken not to misdiagnose such a case as acute overdose if elevated drug concentrations are present. Postmortem cultures may not yield results due to the fastidious nature of certain anaerobic organisms, or they may be non-contributory due to prior antibiotic therapy during hospitalization. Therefore, correlation with antemortem blood or tissue cultures is imperative.

While there are many articles in the clinical literature on the subject of IDU-related clostridial soft tissue infections, a review of the forensic literature showed only one study which discussed necrotizing fasciitis due to spore-forming clostridial agents in connection with BTH. This presents three further cases of a rare but significant association between clostridium species and soft tissue infections in injecting heroin abusers.

#### Heroin, Clostridium, Necrotizing Fasciitis



### H188 Cable-Tie Neck Ligatures: A Series of Five Cases

Nicole B. Ahmed, BA\*, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49007; Laura Bauler, PhD, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49008; Joyce L. deJong, DO, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49008; Jacqueline J. Pender, MD, Miami-Dade Medical Examiner Office, Miami Beach, FL 33139; Theodore T. Brown, MD, Kalamazoo, MI 49008; Joseph A. Prahlow, MD, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49007

**Learning Overview:** After attending this presentation, attendees will understand about occasional deaths that occur in association with cable-tie neck ligatures. Five cases will be highlighted, including a double-homicide, a suicide, and a combined homicide-suicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by drawing attention to the use of cable ties as ligatures for potential strangulation.

Asphyxial deaths occur due to decreased oxygenation of tissues related to depleted environmental oxygen, chemical interference with oxygen utilization, external or internal airway obstruction, trauma or compression resulting in the inability to breathe, or external neck compression, which may involve airway and blood vessel compromise. Most deaths related to external neck compression involve a neck ligature. Such ligature-related cases are typically categorized into two subtypes: hanging, in which the victim's body weight contributes to the compressive force of the ligature, and strangulation, in which the victim's body weight does not contribute to the compressive force of the ligature. While asphyxiation is not an uncommon suicide mechanism, suicide via ligature strangulation is rare because the patient is often unable to maintain tension in the ligature after losing consciousness. Ligature strangulation is much more common in homicides. The use of cable ties as ligatures for strangulation has been reported in the scientific literature and news media, but its occurrence is rare. This study presents a series of five forensic cases in which cable-tie ligatures were discovered about the necks of the decedents.

The first two cases were related to a combined double-homicide/suicide in which two brothers, aged 11 and 15 years, were strangled with industrial cable ties by a family friend with a history of paranoid schizophrenia, who then hanged himself. One of these cases involved a unique mechanism in which the cable tie was not around the anterior neck, but instead was around the anterior chin with marked posterior compression of the mandible and pharynx. In case three, a middle-aged man with a history of alcoholism and depression had an argument with his mother shortly prior to securing two cable ties tightly around his neck. Cases four and five represent a homicide-suicide scenario in which cable ties were found loosely fastened around the decedents' necks. A father and his 6-year-old son were found dead and decomposing in the enclosed compartment of a motor vehicle, the interior of which had been soaked with gasoline and other volatile hydrocarbons. Both succumbed to asphyxia via inhalation of volatile hydrocarbons, with the cable-tie ligatures presumably in place as a back-up mechanism to ensure their deaths.

Intentional self-harm accounts for most asphyxial deaths reported nationally.<sup>1</sup> Although suicide by ligature strangulation is rare, it has been reported in the medical literature.<sup>2</sup> Suicide via strangulation using cable ties has also been reported.<sup>3-5</sup> Despite the presence of several media reports of cable ties used in homicidal strangulations, reporting of such cases in the scientific literature is rare.<sup>6</sup> Suicides employing multiple methods are referred to as "complex suicides."<sup>7</sup> Cases four and five are a situation where the neck cable-tie ligatures represent a presumed planned secondary method for ensuring both deaths.

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#### Cable Ties, Ligature Strangulation, Homicide



**H189 WITHDRAWN**





### H190 It's Not Just Assault Rifles: A Case Study of a Mass Shooting Involving a Shotgun

Brooke H. Blake, MD\*, University of Texas Medical Branch Galveston, Galveston, TX 77555; Erin A. Barnhart, MD, Galveston County Medical Examiner's Office, Texas City, TX 77591

**Learning Overview:** After attending this presentation, attendees will better understand the most common shotgun and its prior use in mass shootings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the less common use of shotguns in mass shootings and providing a review of shotgun ballistics for forensic pathologists. While shotgun injuries are common, death tolls from single events do not generally reach the double digits.

There is no consensus definition of mass shooting; however, the Congressional Research Service defined it as “incidents occurring in relatively public places, involving four or more deaths—not including the shooter(s)—and gunmen who select victims somewhat indiscriminately.”<sup>1</sup>

Since its introduction in 1951, the Remington® Model 870 has become the best-selling shotgun of all time—more than 12 million have been made, and it is used in several different countries by law enforcement, the military, and civilians.<sup>2</sup> With multiple different variants and modifications and cheaper cost, the Model 870 is used in a myriad of different situations: self-defense, clay and field shooting, hunting (deer and fowl), and tactical. These guns normally hold five or seven shells that are easily fed into a bottom-loading chamber to allow for reloading without pausing.

Ten people were killed and 13 injured in a mass shooting incident at a large local high school. The victims were confined to one room and two small closets. The suspect was arrested approximately 29 minutes after the first shot was fired. Police discovered a .38 caliber Rossi® revolver on scene and a Remington® 870 12-gauge short-barreled shotgun that the suspect relinquished on scene. Both were legally owned by a relative. The suspect discarded the Rossi® revolver early in the attack after emptying the chamber and fired all remaining shots from the Remington® 870. The barrel had been sawed off even with the magazine tube, and double aught buckshot (each pellet is a .30 caliber size) was recovered at the scene. The shooter wore a bandolier with the ammunition and reloaded by hand. Police first engaged the suspect within four minutes of the first shot; however, he was able to hold off police with the shotgun only and continue his spree until surrendering to the police without sustaining injury.

At autopsy, shotgun pellets were recovered from each decedent, each within the double aught buckshot caliber. The ammunition used had approximately eight pellets per shot; multiple decedents had more than eight, indicating they had been hit by multiple shots and often in fairly tight configurations.

Out of 143 weapons used in mass shootings (lone shooter in a public place with 3+ deceased) in America from 1982–2012, one public database notes that 71 were semiautomatic handguns (50%), while 21 shotguns were used (15%).<sup>3</sup> This case shows that while semiautomatics are the most commonly used, shotguns can be just as deadly and may be more readily accessible.

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#### Shotgun, Mass Shooting, Ballistics



## H191 Elevator-Related Deaths

Joseph A. Prahlow, MD\*, Western Michigan University School of Medicine, Kalamazoo, MI 49007; Zuhha Ashraf, MD, Los Angeles County Medical Examiner-Coroner, Los Angeles, CA 90033; Natalie Plaza, MD, Los Angeles County Medical Examiner-Coroner, Los Angeles, CA 90033; Christopher B. Rogers, MD, Los Angeles County Medical Examiner-Coroner, Los Angeles, CA 90033; Pamela A. Ferreira, MD, Columbia, MD 21044; David R. Fowler, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223; Melissa M. Blessing, DO, Harris County Institute of Forensic Sciences, Houston, TX 77054; Dwayne A. Wolf, MD, PhD, Harris County Institute of Forensic Science, Houston, TX 77054; Michael A. Graham, MD, Saint Louis University School of Medicine, St. Louis, MO 63104; Kelly L. Sandberg, MS, Franklin County Coroner's Office, Columbus, OH 43201; Krista L. Timm, MD, Denver, CO 80204; Theodore T. Brown, MD, Kalamazoo, MI 49008; Patrick E. Lantz, MD, Wake Forest School of Medicine, Winston-Salem, NC 27157-1072

**Learning Overview:** After attending the presentation, attendees will be aware of risk factors associated with elevator-related injury and death, including factors related to the decedent and those associated with the elevator and surrounding environment, as well as factors connected to other persons' actions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by reviewing multiple deaths associated with elevators, with varying causes and mechanisms of death, occurring in multiple jurisdictions over the past several decades.

Injuries and deaths related to elevators are relatively rare but have been reported in both the medical/scientific literature, as well as the popular press.<sup>1-4</sup> Mechanisms of injury/death include asphyxia-related injuries, Blunt Force Injuries (BFI), such as those resulting from crushing trauma and falls from heights, avulsion types of injuries, and environmental injuries. Decedents include elevator maintenance personnel, construction workers, elevator users, and those involved in risk-taking behavior. Occasional natural deaths and non-elevator-associated traumatic deaths also occur on elevators.

This study reports on 57 elevator-related deaths, occurring in 8 different jurisdictions, and encompassing cases occurring over the past 40 years. The series of cases does not include every elevator-related case occurring in each of the jurisdictions during the listed time-frame. Therefore, frequency data cannot be calculated based on this study. Rather, the collection of cases represents an attempt to gather a wide variety of case types, to gain an understanding of how such deaths occur, and how elevator-related injuries and deaths may be avoided. In addition to an overall review of the cases, select cases will be highlighted to emphasize important forensic considerations as well as preventive strategies.

Of the 57 cases included in this review, 20 had causes of death which could be categorized as BFI related to falls, 14 could be categorized as traumatic asphyxia, 10 involved crushing types of BFI, 5 involved other blunt force trauma, 1 involved complications of spine trauma, 1 case involved decapitation, 2 involved drowning, and 3 were electrocutions. In one case, a cause of death could not be determined secondary to decomposition. The manner of death in all but two cases was certified as accidental. There was one suicide and one undetermined.

Several important potential preventive strategies are highlighted by the series of cases. For maintenance and construction workers, as well as the general population, elevators, elevator shafts, and all the mechanical and electrical aspects related to them, must always be recognized as potentially dangerous, especially when they are under construction, under repair, or when they are not functioning normally. Constant vigilance and awareness around elevators is necessary to avoid potentially life-threatening accidents. For the general population, suggested strategies to avoid injury and death include the following: do not use improperly functioning elevators; report malfunctioning elevators immediately; do not attempt to forcibly enter or exit an elevator; do not enter or exit a "stuck" elevator; do not attempt to repair a malfunctioning elevator; do not enter an elevator shaft; do not "play" on elevators or in elevator shafts. For construction and maintenance workers, the recognition that elevators are potentially dangerous is even more important, as the nature of work in and around elevators places such persons at greater risk of injury and death. Employing appropriate safety devices, precautions, and protocols can help to avoid accidental injury and death. Examples include wearing safety harnesses; shutting down elevator function while working above, below, or near elevators; and following all industry safety regulations.

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### Elevator, Death, Forensic



**H192 WITHDRAWN**



## H193 Tractor-Related Fatalities

Tracy C. Johnson, BS, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49007; Abigail Jacqueline Grande, MPH, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49008; Amanda O. Fisher-Hubbard, MD\*, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49007

**Learning Overview:** After attending this presentation, attendees will be more familiar with the variety of environmental and occupational settings in which tractor-related fatalities can occur, as well as trends in certification of these deaths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by quantifying the frequency of physical injuries associated with tractor-related fatalities and examining the relationship of these traumatic injuries to underlying medical conditions, which can aid forensic pathologists in elucidating the medically relevant contributions to a given fatality.

Tractor-related fatalities remain a significant cause of preventable deaths, despite governmental attempts to reduce their prevalence. Approximately half of all agricultural fatalities are associated with farm tractor accidents.<sup>1</sup> The rural setting of many of these accidents also results in delayed transportation and treatment; the majority of deaths occur at the site of the accident.<sup>2</sup> These fatalities can vary widely by mechanism of death, contributions from underlying natural disease, environmental settings, and affected population. The majority of tractor-related fatalities involve overturned tractors, also known as tractor “rollovers.” The most common injury pattern leading to death in these fatalities is crushing of the chest from the weight of the overturned tractor. While tractor-related fatalities most commonly affect farmers, a significant portion of these deaths affect other occupations, including construction and manufacturing workers, military personnel, students, and others.<sup>3</sup>

This study hypothesizes that most tractor-related fatalities are accidental deaths and that most of these accidents include a component of asphyxia.

For the purposes of this study, a “tractor” is considered a four-wheeled or tracklaying automotive vehicle used especially for drawing heavy equipment; this also includes riding lawn mowers. This study examined a series of cases in which death occurred in the setting of tractor operation. To capture all tractor-related deaths, a search was conducted using multiple methods, including searching for the term “tractor” in the investigator narrative of the investigative report, in the *Vehicle Type* of the investigative report, and in the *Injury Description* of the death certificate. A search was also conducted for the term “lawn” in the *Injury Description* of the death certificate. This study includes cases investigated by medical examiners across multiple counties in Michigan from 2008 to 2018.

A total of 126 potential tractor-related cases were identified using the above search techniques. Of these 126 cases, 35 met the criteria for the purpose of this analysis. These decedents ranged in age from 1 to 90 years, with mean and median ages of 61 and 63 years, respectively. Only four (11%) of the 35 decedents were female. The majority of these deaths (77%) were certified as accidental; this study also located two (6%) suicides and six (17%) natural deaths related to tractor operation. Four of the deaths occurred at work, while 26 were not at work, and the remaining five deaths were unknown. Only two of the decedents were not the operator of the tractors at the time of the incidents; this included a 2-year-old passenger and a 1-year-old pedestrian. Of the 29 non-natural deaths, eight involved an overturned tractor causing blunt force injury and/or asphyxiation of the operator. Six of these 29 deaths involved a tractor versus a moving vehicle, including a snowmobile versus tractor, a moped versus tractor, and four lawn tractors versus cars. The remaining non-natural deaths included three fallen tree branches, three falls from the tractor, three carbon monoxide poisonings, two drownings, two pedestrians run over by the tractor, an entanglement in the tractor machinery, and a farm animal attack.

As hypothesized, the majority of tractor-related fatalities examined were accidental deaths. Notably, a minority of these accidental deaths involved asphyxia due to an overturned tractor. To conclude, tractor-related fatalities include a wide variety of causes of death, often occur outside of an occupational setting, and involve a wide age-range of decedents.

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### Tractor, Lawn Mower, Traumatic Asphyxia



### H194 Postmortem Computed Tomography (PMCT) in Shooting Fatalities

*Marloes E.M. Vester, MD\**, Netherlands Forensic Institute, The Hague, ZH 2497GB, NETHERLANDS; *Rick R. Van Rijn, PhD*, Amsterdam University Medical Centers, Amsterdam 3544MT, NETHERLANDS; *Chandra Y. Gerrard, BS*, University of New Mexico, Department of Radiology, Albuquerque, NM 87106; *Kurt B. Nolte, MD*, Office of Medical Investigator, Albuquerque, NM 87131-0001; *Reinoud D. Stoel, PhD*, Laan van Ypenburg 6, Den Haag, NETHERLANDS; *Gary M. Hatch, MD*, University of New Mexico, Department of Radiology, Albuquerque, NM 87106

**Learning Overview:** After attending this presentation, attendees will understand the accuracy of PMCT compared with autopsy and PMCT in fatal cases of firearm injuries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing guidance concerning the use of PMCT in fatal cases of firearm injury.

PMCT has become integrated into the evaluation of decedents in some United States medical examiner offices and in medicolegal offices in several other countries. This study investigated the value of a total-body PMCT scans in firearm injury-related deaths.

This retrospective medicolegal autopsy case series was performed using data from a statewide, centralized, academically based, United States medical examiner's office. Firearm injury deaths that occurred between January 1, 2016, and December 31, 2016, were reviewed. Only cases with a total-body PMCT and a total-body autopsy were included.

Autopsies were performed and documented by unblinded board-certified forensic pathologists acquainted with the PMCT results. PMCT results were reevaluated by a forensic radiologist, blinded for autopsy results, and scored by body region (head-neck, thoracoabdominal, and extremities). Injuries for these body regions were scored as absent, minor, or major. The PMCT injury scores per body region were compared to autopsy results with McNemar's test. An exemplary Bayesian network was built with HUGIN® to help visualize the determinative value of these results.

Out of the annual 7,000 cases investigated at the Office of the Medical Investigator (OMI), 376 had firearm injuries. From these identified cases, 247 cases were excluded due to a lack of a total-body PMCT and/or a total-body autopsy, or the presence of disruptive surgery, an old firearm injury, or a long postmortem interval with the resulting decomposition.

Based on the final autopsy reports, a total of 129 cases with firearm injuries remained, of which 47 were suicides, 80 homicides, 1 case was undetermined, and 1 case was determined as accidental. The decedents consisted of 104 males and 25 females, aged 3-83 years old (median age 35 years). Based on PMCT results, 102 (79.1%) cases had injuries relevant to the cause of death in a single body region, with 51 cases having injuries in the head-neck region, 50 cases in the thoracoabdominal region, and 1 case having a single injury of the leg. The remaining 27 cases had injuries in more than one region. Additionally, in 96 of the cases, the bullet or fragments of the bullet were retained.

When comparing the total-body autopsy results with the total-body PMCT results, for those 102 cases with single body-region injuries relevant to the cause of death (thus based on PMCT), 12 potentially relevant injuries (cardiac and major vessel diseases and arm injuries) outside the particular body region, were additionally identified by autopsy. These 12 injuries were in 12 different cases, of which 5 were in the head-neck region group and 7 in the thoracoabdominal group. Yet, in all 12 of these cases with missed injuries, there was evident firearm injury to the body, also noted by PMCT. It is believed that it is unlikely these additionally identified abnormalities by autopsy contributed to the death. In cases where more than one region were injured, autopsy performed better in identifying injuries. Per this study, it is believed that PMCT can accurately identify the key injuries in fresh decedents with firearm injuries, but without surgery, for nearly all cases in which a single body region was injured.

#### Autopsy, Computed Tomography, Gunshot Wound



## H195 Jay Dix Day Lecture Series 2019

*Michael A. Graham, MD\**, Saint Louis University School of Medicine, St. Louis, MO 63104; *Joseph A. Prahlow, MD\**, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49007; *James R. Gill, MD\**, Office of the Chief Medical Examiner, Farmington, CT 06032; *Jonathan Hayes, MD\**, Office of the Chief Medical Examiner, New York, NY 10001; *Andrew M. Baker, MD\**, Hennepin County Medical Examiner, Minneapolis, MN 55415; *Joyce L. deJong, DO\**, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI 49008; *Tracey S. Corey, MD\**, Louisville, KY 40207

**Learning Overview:** A proper medicolegal death investigation is a multidisciplinary process that often involves non-medical personnel as well as medical professionals. This annual series of lectures is intended to provide the non-forensic pathologist forensic scientist a comprehensive basic review of selected topics in forensic pathology to increase familiarity and understanding and enhance inter-discipline communication.

This year's lecturers will discuss the medicolegal investigation of deaths related to environmental conditions; deaths due to asphyxia; the investigation of suicide; deaths due to firearm injuries; and deaths related to sports. After attending this presentation, attendees will understand how and why deaths related to the previously specified topics occur and will learn a systematic approach to the evaluation of such deaths that can easily be implemented in their daily practices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a comprehensive review of what causes and contributes to deaths related to the previously specified topics. Attendees will be able to systematically evaluate deaths that they encounter in their daily practices in which the previously specified topics may have played a role.

There are multiple factors that can play a role in deaths that are related to the environment. This lecture will review human and environmental factors that potentially affect a person's ability to survive under a variety of environmental conditions. What constitutes "hostile" environmental conditions, how humans attempt to adapt to changes in the environment, and what prevents successful adaptation to changing environmental conditions will be discussed. There will be discussion on how humans get into hostile environments, how they attempt to adapt to these conditions, and what factors prevent adaptation and/or escape from inhospitable environments. Deaths caused or contributed to by cold, heat, altitude, drowning, animals, and lightning will be among the topics discussed.

Human life requires the uptake and utilization of oxygen along with the release of metabolic waste. Failure of these processes leads to asphyxia. Proper evaluation of asphyxial deaths requires knowledge of the entities that cause asphyxia, pathophysiologic mechanisms, asphyxia death scenarios, and factors that contribute to death. This lecture provides comprehensive discussion of the investigation of deaths that may involve asphyxia.

Suicide can be accomplished in many ways, some overt and others covert. The accurate recognition of suicide has important implications for the decedent's survivors, estate, and others. Unlike most other manners of death determinations, an assertive determination of the decedent's intent is fundamental in certifying a death as suicide. The proper investigation of a suicidal death may offer insight into the motivation for the death and provide information in the development and implementation of preventive strategies. This lecture will comprehensively discuss the investigation and interpretation of findings in suicidal deaths.

Firearm fatalities are a major cause of non-accidental morbidity and mortality in the United States. Multiple factors and mechanisms are involved in producing firearm injuries. Understanding and evaluating firearm injuries requires a basic understanding of how these injuries are produced and how to distinguish them from other types of trauma. This lecture will provide a comprehensive review of these issues.

There are multiple factors that can play a role in deaths that are temporally related to participating in and, occasionally, being a spectator at sporting or other recreational activities. This lecture will review several issues that arise in the context of investigating deaths that occur in relation to sports/recreational events. Understanding factors that are involved in these deaths also helps in instituting safety measures to protect participants and spectators.

The death of an apparently healthy infant is a devastating event for the infant's survivors and is accorded significant attention by society. Infant death may be caused by a wide variety of diseases and injuries. Accurate recognition of the cause, mechanism, and manner of death has important implications for the survivors, other interested investigative and health agencies, and society in general. Recognition of factors involved in sudden unexpected infant deaths can help in enhancing the safety of other family members and serve as a basis for formulating death-prevention strategies. This lecture will discuss the investigation and interpretation of findings in sudden unexpected deaths involving infants.

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### Medicolegal, Death Investigation, Forensic Pathology



### II Closing Judicial Psychiatric Hospitals: Not Guilty by Reason of Insanity Offenders' Socially Dangerous Treatment Inside the Psychiatric Community Network—An Italian Perspective

Felice F. Carabellese, MD\*, University of Bari, Bari 70124, ITALY; Luca Castelletti, MD, USL, Toscana, ITALY; Franco Scarpa, MD, USL, Toscana, ITALY; Donatella La Tegola, PhD, University of Bari, Bari 70124, ITALY; Roberto Catanesi, MD, Bari 70124, ITALY

**Learning Overview:** After attending this presentation, attendees will better understand the treatment practices of socially dangerous offenders inside the psychiatric community network called REMS (Residence for the implementation of Security Measures).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the security measures that the new national residential network is developing to share common good practices of care.

Two years after the introduction of Italian forensic psychiatric reform, the new national residential network for subjects in security measures is now trying to develop and share common good practices of care, according to the contents of the new legislation. In this work, progressive steps of assessment and care of those admitted to the REMS will be illustrated, as well as the way new scenarios may impact the role of expert judgement in the courts and their effects on forensic subjects' referrals. Some critical points fostered by the new system, including criteria for admissions and clinical rationale for releases to lower levels of security, are discussed further in this work.

Regarding risk issues, the forensic and general psychiatric network may benefit from an integration with recovery-oriented models, looking at the risk-taking paradigm and strength model.<sup>1</sup> Concept of risk, in this manner, is viewed as life challenges in prosocial goals, real social integration, autonomy, advocacy, and the protection of rights.<sup>2</sup> The combination and integration of both concepts of risk, according to a patient's features, his environment, service provision, and quality of networks may better suit the specific institutional and cultural Italian ground for security measures.

Inclusion of different ways of conceiving risk may foster a patient's active involvement into pathways of care and a more active participation of staff members to care programs as they better identify themselves as mental health staff members, despite also being custodial agents. The closing of Judicial Psychiatric Hospitals (OPGs) has put the duty of adopting evidence-based strategies of violence risk assessment functionally at the development of risk management and caring strategies.<sup>3,4</sup>

It is a priority to introduce the daily practice of using reliable instruments of violence risk assessment, bearing in mind that they have statistical limits and their use may present side effects in terms of prolonged hospitalizations.<sup>5,6</sup>

In many regions, and at the national level, an agreement is requested to promote quality networking of the main actors and the interested stakeholders. The juridical system (i.e., courts and surveillance judges), community psychiatric services, lawyers, forensic experts, and social services work together with the goal of developing shared practices to provide effective assessments and regulations.

The closure of forensic hospitals represents a valid opportunity for Italian psychiatry to plan and allocate proper resources to sustain this reform, including training and education for all professionals involved in the processes of care.

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#### REMS, Risk Assessment, Forensic Psychiatric Treatment



## Psychiatry & Behavioral Science – 2019

### I2 The Hearsay Rule: An Adversary to the Mental Health Field—Which Field Is Next?

Amber N. Bridges, MD\*, LAC+USC Institute of Psychiatry and Law, Los Angeles, CA 90033

**Learning Overview:** After attending this presentation, attendees will understand: (1) how a recent California case impacts the admissibility of hearsay material relied on by expert witnesses, (2) how the case is restricting an expert's ability to use collateral material informing their opinion when testifying in involuntary civil commitment cases, and (3) how such restrictions may hinder mental health treatment and, ultimately, the patient.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of how an expert's use of collateral sources has been generalized and applied beyond that of the original landmark case.

In the 2016 case, *People v. Sanchez*, the defendant was convicted of multiple criminal felonies with gang enhancements after a gang expert witness opined that the defendant was likely a member of the Delhi gang.<sup>1</sup> The expert did not interview the defendant and based his opinions on collateral information. The defendant contended the expert's testimony on grounds of hearsay, which inspired the California Supreme Court to revisit the proper use of the hearsay rule. The Court ruled that "when any expert relates to the jury case-specific out-of-court statements and treats the contents of those statements as true and accurate to support the expert's opinion, the statements are hearsay." Consequently, the Court reversed the defendant's gang enhancement charges.

According to California Evidence Code § 1200, (The Hearsay Rule), "hearsay evidence is evidence of a statement that was made other than by a witness while testifying at the hearing and that is offered to prove the truth of the matter stated. Except as provided by law, hearsay evidence is inadmissible." Prior to the *Sanchez* ruling, California courts generally allowed experts to testify using hearsay statements on the basis that they were used to assist the expert in forming an opinion and not to be used as true facts by the trier-of-fact. Post-*Sanchez*, many courts are no longer permitting testifying experts to use hearsay information. This includes mental health experts, who because of this ruling, are prohibited from using data obtained from a patient's treatment team or family members when testifying at civil commitment writ or conservatorship hearings.

Mental health providers have the unique challenge of treating patients who are frequently unable or unwilling to provide necessary information due to symptoms of their mental illness. It is common practice for mental health experts to rely on statements from a patient's family members regarding the patient's medical history, observed symptoms, and changes in functional status. Collateral information from family members is often a crucial aspect of clarifying a patient's diagnosis, as well as vital to constructing an appropriate disposition plan for the patient; particularly when the patient has been reliant on family members for housing and financial support. The absence of such vital information could be markedly detrimental to the mental health expert's testimony and risks depriving the trier-of-fact from gaining a thorough understanding of the issue being addressed.

Frequently, mental health experts rely on relevant third-party information, in addition to direct patient assessment, as a basis for forming clinical opinions. Barring such information during expert testimony may limit the expert's ability to sufficiently prove the need for continued involuntary hospitalization or conservatorship.

Other challenges include the feasibility of requiring multiple witnesses to testify in court rather than allowing the expert witness to present statements on their behalf. The financial consequences of having each witness with relevant information (including, but not limited to, multiple health care providers, law enforcement officers, and patients' family members) taking time away from their duties to testify in court may not be reasonable. There is also the risk of potential witnesses, such as family members and treatment providers, damaging their relationship with a patient when testifying about them in court. A case example that illustrates some of the repercussions of the *Sanchez* ruling's application to the mental health field will be presented. A discussion regarding how this ruling could eventually impact other fields of expertise will also be addressed.

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<sup>1</sup>. *People v. Sanchez*, 63 Cal.4<sup>th</sup> 665 (2016).

#### Hearsay, Mental Health, Expert Witness





### I3 The Art of Manliness: Law, Gender, and Sexuality in a 19th-Century Case Study

*Annarita Franza, PhD\**, Department of Experimental & Clinical Biomedical Sciences, University of Florence, Florence, ITALY; *Vincenzo Lusa, JD\**, Rome 00151, ITALY

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**Learning Overview:** After attending this presentation, attendees will understand the role of jurisprudence in the investigation of sex and gender issues in individuals with Disorders of Sex Development (DSD) (i.e., congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting a multidisciplinary framework based on the analysis of a 19<sup>th</sup>-century case study that will demonstrate the impact of jurisprudence in identifying the biological sex, gender identity, and social-sex role in a person with sex or gender non-conformity.

Carmela C. was born in 1880 in Castelvetro, an Italian medieval village near Trapani. When she was in her 20s, her parents, Caterina and Antonio, took her to the Civil Hospital in Palermo because she had not yet had her period. Here, Carmela was examined by the physician Saverio Tomasini who diagnosed multiple genital anomalies. Moreover, Tomasini conducted a lengthy interview with Carmela at which she confessed to having sex with female partners and feeling her gender was masculine. Apart from her physical aspect, Carmela was a charming girl with long black hair. Carmela did not recognize herself as a woman; attending to any female activities or getting married to a man disgusted her. She desired to be a coachman and meet the girl of her dreams. So, Carmela was more than glad when Tomasini told her she was not a girl. She had genital reconstructive surgery and masculinized her name in Carmelo. The physician Giuseppe Castellana, who wrote a scientific paper on this case, operated. In the years that followed, Carmelo became a coachman and a husband.

This unique case was the occasion for Tomasini to rethink the role of legal medicine and jurisprudence in the assignment of sex in newborns with DSD. He confronted the Italian Civil Code in a transnational perspective with the French and German Codes and intensely discussed Art. 374. Tomasini analyzed the Prussian Criminal Code in which sex reassignment is highly recommended in infants with ambiguous external genitalia after 18 years of age in the presence of a medicolegal expert who would certify the conformity of the patient's anatomical and psychological sex in keeping with his/her status and gender role (Art. 19-23). Tomasini thus proposed a review of Art. 374, including a medical certification of "uncertain sex" for newborns with DSD. He then analyzed Art. 105, 106, 107, and 112 of Italian Civil Code studying the relationship between DSD, marriage, and divorce. Tomasini's analysis underlined the issue of legally recognizing a "third gender," a process started in 2013 when Germany allowed an "indeterminate" gender option on birth certificates. In 2014, both Australia and India permitted the registration of a person's sex as "non-specific." Even as Lesbian, Gay, Bisexual, Transgender (LGBT) rights in Italy have evolved significantly over the course of the past years, DSD persons may still face some legal challenges because of incomplete legislation.

In conclusion, DSD presents a unique challenge regarding medicolegal management; increasing the focus on forensic sciences will help to protect a person's sexual identity beyond stereotypes and prejudices.

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#### Disorders of Sex Development, Case Report, Intersex



### I4 Use of the Minnesota Multiphasic Personality Inventory®-2 (MMPI®-2) and Rorschach Test in Forensic Psychological and Psychiatric Evaluations Regarding Parenting in the Context of Marital Conflicts

*Ignazio Grattagliano, PsyD\*, University of Bari, Bari 70124, ITALY; Romy Greco, PsyD, University of Bari, Bari 70124, ITALY; Anna Convertini, University of Bari Italy, Department of Psychology, Bari 70124, ITALY; Giancarlo Di Vella, MD, PhD, University of Torino, Department of Public Health Sciences, Torino 10126, ITALY; Valeria Affatati, PhD, University of Bari Italy, Bari 70124, ITALY; Lucrezia Cavallo, PhD, University of Bari Italy, Bari 70124, ITALY; Filippo Campobasso, PhD, Bari 70124, ITALY; Luciana Lo Bianco, PhD, University of Bari, Department of Psychiatry, Bari, ITALY; Ylenia Massaro, PhD, Bari 70124, ITALY; Valentina Stallone, PhD, Bari 70100, ITALY; Roberto Catanesi, MD, Bari 70124, ITALY*

**Learning Overview:** The goal of this presentation is to reinforce the importance of correctly using reliable tests to make accurate assessments for couples in conflict who undergo evaluations in the forensic field by the courts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of correctly using reliable tests to make accurate assessments of couples in conflict who undergo evaluations in the forensic field by the courts.

Evaluation of parenting skills and competencies is an interdisciplinary activity. It involves various subjects, including adults, minors, and families, in addition to the contexts to which they belong. These assessments appear to be even more decisive and important when unresolved conflicts and tensions occur between couples who have children. Such situations require the intervention of judicial authorities, keeping in mind the best interests of the children involved. Given the sensitivity and importance of these assessments, which also assume a prospective profile for the decisions that judges make, it is essential that psychologists and psychiatrists, appointed by magistrates and lawyers, carry out such evaluations by applying scientific protocols recognized by the scientific communities at national and international levels.

The MMPI®-2 and Rorschach tests are the most widely used psychodiagnostic tools in the world as personality assessment instruments and guarantee great scientific reliability; they are instruments that are widely used in many studies all over the world.

The hypothesis of this study is that couples in highly turbulent relationships who involve their children in their conflicts create many psychological problems for them. The higher the level of conflict, the higher the scores on psychodiagnostic indices of the two tests should be.

**Methods:** Fifty-nine couples who had come to the attention of ordinary courts and tribunals for minors were evaluated in Italy: 118 people in total. All Rorschach and MMPI®-2 tests that were administered to both members of these couples were examined. During the appraisals, two different tests were used—the MMPI®-2 and the Rorschach—to better guarantee higher scientific objectivity measurements by crossing the psychodiagnostic data of two different instruments, which are equally valid in personality assessment.

**Results:** Parental suitability: (1) in 8% of cases, parental suitability was evaluated as fully present both in the father and the mother; (2) in 11% of cases, parental suitability was considered fully present in the mother, and deficient but recoverable in the father; (3) in 5% of cases, parental suitability was fully present in the father, and deficient but recoverable in the mother; (4) in 2% of cases, parental suitability was a recoverable deficit in the mother, but in contrast, not recoverable in the father; (5) in 7% of cases of parental suitability, there was a recoverable deficit in the father, but not recoverable in the mother; (6) in 50% of cases, parental fitness was assessed as deficient but recoverable for both the mother and father; (7) in 3% of the sample, mothers were assessed as deficient but recoverable, but not evaluative in the father (i.e., the inability to evaluate derives from the subject being removed from the administration of psychodiagnostic tests, which were experienced as persecutory); and (8) in 14% of cases, at the outcome of the appraisals, the judges evaluated the situation of conflict and violence in the family, in which the children were also involved, to be so serious as to suspend parental authority of both parents and to award custody of the children to other people or to place them in communities for minors.

The initial hypothesis of this study was confirmed. Most conflicted couples had high scores on the MMPI®-2 test relative to the clinical scales of social/relational isolation, of suspiciousness, the scales that signal manipulative behavior. Regarding the Rorschach test, on the other hand, the psychodiagnostic indices most often represented in the most highly conflicted couples concerned the area of psychosocial adaptation, those of relational problems, and those of difficulties in controlling emotions and impulses.

**Conclusion:** If correctly used, the MMPI®-2 and Rorschach tests are confirmed as excellent tools for conducting measurements and evaluations in relation to expert activities concerning the evaluation of competence and parenting skills.

#### Marital Conflicts, Tests, Courts



## I5 Aging and Prison: A Study of a Large Number of Italian Penitentiaries

*Ignazio Grattagliano, PsyD\*, University of Bari, , Bari 70124, ITALY; Carlo A. Romano, University of Brescia, Department of Jurisprudence, Brescia 25122, ITALY; Luisa Ravagnani, PhD, University of Brescia, Department of Jurisprudence, Brescia 25122, ITALY; Silvio Tafuri, Department of Biomedical Sciences and Human Oncology, Bari 70100, ITALY; Francesco Paolo Bianchi, PhD, Department of Biomedical Sciences, Bari 70100, ITALY; Carlo P. Campobasso, MD, PhD, University of Campania, Napoli 80138, ITALY; Graziamaria Corbi, PhD, Campobasso 86100, ITALY; Patrizia Gasparo, University of Bari, Department of Psychology, Bari 70100, ITALY; Roberto Catanesi, MD, Bari 70124, ITALY*

**Learning Overview:** The goal of this presentation is to increase awareness of the condition of elderly prisoners in prison. With careful consideration of the complexities and difficulties that exist within the Italian prison system, and in full compliance with national and supranational frameworks, this study was conducted with the goal of surveying the conditions of elderly prisoners.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of the awareness of the condition of elderly prisoners in prison.

It is well known that elderly people are more vulnerable when compared to younger adults within the same community because of their advanced age. A precise age limit is not implied here, although 65 years is considered the starting point of old age. Therefore, vulnerability is one of the characteristics of the elderly. Such individuals may need health care services, social assistance, and/or protection by the community due to any number of problematic situations and limitations, including illness. Such people may be unable to take care of themselves and are unable to protect themselves from significant harm, exploitation, abuse, violence, and other risks.

Vulnerability and fragility are distinct concepts. Fragility is an accumulation of deficits in the physical, social, and psychological spheres and manifests itself throughout the full continuum of life, regardless of age. Vulnerability is an accumulation of deficits in terms of relative risk and scarce resources. Individual, situational, health, behavioral, resource, biological, and environmental factors can influence fragility. Fragility domains form a triad: (1) physical, (2) psychological, and (3) social. The presence of all three renders a person fragile and puts them at risk for adverse events. Without a doubt, physical frailty can be influenced by psychological and social fragility. For these reasons, this research's hypothesis is that prisoners over 65 years of age manifest difficulties and hardships in adapting to the detention regime, which are, in many ways, both different and more numerous when compared to other adult prison inmates. Interventions aimed at relieving such problems must keep in mind both the obligation of the prisoners' incarceration and atoning for their crimes, while at the same time protecting the rights of these individuals, especially because they are older and therefore generally more vulnerable. One possible alternative could be granting probation and employing alternative measures of detention.

**Methods:** This study distributed a detailed questionnaire to over 100 prisoners who are currently serving prison sentences in both Northern and Southern Italy (the regions of Lombardy and Apulia). The questionnaire concerned personal data, characteristics of the crime committed, imprisonment, pre-existing psychophysical health conditions and subsequent incarceration, family news, and other information related to the execution of the sentence.

**Results:** As far as the crimes committed are concerned, the most frequent are offenses related to the participation in mafia-style organizations, white-collar crime, particularly heinous crimes, long criminal careers, financial and administrative offenses, and related offenses, as well as the trafficking and marketing of narcotic substances. Psychophysical health conditions and the adaptation to prison often turn out to be precarious.

**Conclusions:** The increased percentage of the elderly prison population is a worldwide phenomenon and is certainly linked to a complex causality. The main reasons for this phenomenon are: demographics, legal issues, state of health, inappropriate response to social changes, reduction of psychiatric and geriatric care, and lower capacity for reintegration of the senior prisoner

The basic question appears to be: Are Italy's prison facilities, which were originally designed to host and contain young, healthy prisoners who do not suffer from chronic diseases, suitable for receiving elderly prisoners who suffer from disabilities and chronic diseases? The extreme passivity of life during detention most likely has a negative effect on the psychic and cognitive state of the elderly prisoners. Furthermore, many of these inmates suffer from psychiatric pathologies and need treatment, re-education, and social/healthcare assistance, which are different from the needs of the rest of the adult prison population.

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### Elderly, Prison, Illnesses



### I6 Assessing the Risk of Violence for Psychopaths: What Is the Right Method?

*Sebastien S. Prat, MD\*, St. Joseph's Healthcare, McMaster University, Hamilton, ON L8N 3K7, CANADA; Gary A. Chaimowitz, MD, Saint Joseph's Healthcare, McMaster University, Hamilton, ON L8N 3K7, CANADA*

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**Learning Overview:** After attending this presentation, attendees will understand the relationship between psychopathy and violence and assessing the level of risk within this population.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping identify the issues when assessing psychopathy.

Psychopathy is a common diagnosis made when assessing violent offenders. The Psychopathy Checklist-Revised (PCL-R) is the gold standard tool to assess the presence of this diagnosis. Newer tools have been developed, such as Psychopathy Personality Inventory, but this is not commonly applied to the forensic context at this time. However, assessing psychopathy does not equal assessing risk, and psychopathy needs to be assessed by trained clinicians. The development of the assessment tools has evolved to identify the criteria that accurately define this pathological construct.

Why is psychopathy so often associated with the risk of inappropriate behavior? Most of the research has been conducted in forensic settings. Moreover, some studies focused on the criminal behavior rather than the personality disorder. According to some research, the antisocial item of the PCL-R, which is associated with violent behavior, misidentifies the antisocial behavior of some individuals with psychopathy. Some suggest that we should differentiate between psychopathy and PCL-R-diagnosed psychopathy, although at this time, PCL-R is considered the only reliable tool. The other issues that are identified when assessing the risk of violence is related to the fact that high scores on the PCL-R are not associated with high risk. Therefore, using other risk assessment tools is necessary to accurately identify the risks; some of them have an item directly related to the PCL-R, but include many more aspects that allow a broader assessment.

Some clinicians continue to use psychopathy as the sole method to identify risk without appreciating the difference between diagnosis and behavior. Besides identifying the key aspects of psychopathy and risk of violence, this presentation will be an opportunity to present the legislation of the Dangerous Offenders in Canada. Under some specific circumstances, an individual may be qualified a Dangerous Offender and be sentenced to jail time for life. An alternative may be to be qualified as a Long-term Offender which will impose 10 years of probation after being released from custody.

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#### **Psychopathy, Violence, Assessment**



## I7 A Psychopathologic and Etiologic Analysis of Psychopathic Traits in Klinefelter Syndrome

Raphaëlle Jouin\*, Rennes, Bretagne 35000, FRANCE

**Learning Overview:** The goal of this presentation is to provide an understanding of psychopathic traits in Klinefelter syndrome and to analyze their etiologic and psychopathologic components.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the general comprehension of genetic and epigenetic mechanisms in psychopathy.

Klinefelter Syndrome (KS) (also known as 47, XXY) is the most common aneuploidy (1/650) of sexual chromosome abnormalities among males (0.1% to 0.2% of the male population). This syndrome is largely underdiagnosed because of a large phenotypic variability. Physical (tall, sparse hairiness, gynecomastia), hormonal (hypogonadism with low testosterone levels), and psychological symptoms (normal to low average total Intelligence Quotient (IQ), low verbal IQ, various cognitive symptoms) are commonly described, as are high levels of psychiatric comorbidities, including aggressiveness. Higher risks of committing sexual crimes and arson (compared to criminal controls) was recently depicted.<sup>1</sup> However, because of the small prevalence of persons involved, only a few studies provide quantitative or qualitative robust results on forensic populations.

A small sample of KS, including three male adult inpatients presenting aggressive or violent symptoms or criminal history, were recruited in Belgium and France to assess their psychopathic traits using the Psychopathy Checklist-Revised (PCL-R) of Hare.<sup>2</sup> First, results showed weak scores on facet 1 (interpersonal). However, scores assessing affective traits (facet 2), lifestyle, especially impulsivity (facet 3), and antisocial traits (facet 4), as well as item 11 (promiscuous sexual behavior) seemed higher.

Results could be related to characteristic genetic features with consequences on neural development (limbic system and temporal lobe abnormal cortical volume, hemispheric specialization shortcoming). These involve alterations of both complex cerebral (attention, empathy), and behavioral regulation functions (inhibition, mental flexibility, emotional response modulation, control of own actions).<sup>3,4</sup>

Interestingly, some similar findings also underlie current etiologic and psychopathologic hypotheses of psychopathy.<sup>5,6</sup> Nevertheless, double diagnosis is scarce among KS. In addition, some KS features are antagonistic with those hypotheses (e.g., testosterone levels) without prohibiting clinical psychopathic traits among KS.<sup>7,8</sup>

Given these discrepancies, the well-documented syndrome of KS, which allows reasoning on both genetic and epigenetic mechanisms, also introduces the concepts of adaptability and neuronal ecology, which may clarify murky interactions of biological, psychological, and social factors entangled in the development of psychopathy.

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### Klinefelter Syndrome, Psychopathy, Psychopathology



### **I8 Aggression Is Inversely Related to Serum Cholesterol**

*John L. Young, MD\*, Yale University, New Haven, CT 06511-4048*

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**Learning Overview:** After attending this presentation, attendees will understand the correlation between lower cholesterol and more aggressive behavior, which will be helpful in situations in which this correlation may be important.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by focusing on the knowledge and understanding of the subject of cholesterol levels and rates of violence. Attendees will broaden and deepen their knowledge of an important arm of the physiological chemistry that underlies human aggressive behavior, whether it is “accidental” or against self or others. This will make attendees better ethics consultants and expert witnesses. This presentation will advance the field of forensic behavioral science as well by further extending and strengthening the scope of potential new treatments for aggression as well as enhanced preventive measures.

Most clinicians across all fields of practice today would consider it a mark of thoroughness and even of excellence in their performance as practitioners when they do not omit checking their patients’ serum cholesterol, no matter what the purpose of the office visit may have been. Clinicians base this practice on the clinically demonstrated connection between control of cholesterol levels and good cardiovascular health. It is a clinical impression that enjoys a strong base in established theory.

At the same time, it is becoming gradually clear that another clinical correlation of well-controlled serum cholesterol concentration is likely to be in play. A growing number of observers are reporting associations between lowered cholesterol levels and increased serious violent behavior. This unexpected result naturally raises questions well worth answering through appropriate queries. Some would suggest that the finding is pure coincidence. Others consider the relationship likely to be merely an indirect one, that the actual operating factor happens to be linked to either the lowered cholesterol or to the increased aggression. Examples include an endocrine phenomenon or epiphenomenon, chemical or structural resemblances suggesting as they can significant functional relationships. This may come about through shared metabolic pathways that have varied status among different groups of investigators.

Unlike the connection of lower serum cholesterol with better cardiovascular health, the phenomenon of its correlation with more violent behavior currently lacks a widely accepted theoretical explanation. Such an absence is a matter of serious concern since the safety of innocent persons is at stake. Ethics expertise is of unusually serious importance since several of the competing values in this case are of such heavy weight. Also, they could turn out to be considerable in number, depending on the details of the investigation that might be proposed or on the clinical or legal decision to be made. In no particular order, the concerns or values may, among others, include beneficence, non-maleficence, autonomy, privacy, safety, and justice

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#### **Cholesterol, Violence, Accidents**



## I9 Serotonin and Aggression in Children and Adolescents: A Systematic Review

R. Ryan Leahy, MD\*, Charleston, SC

**Learning Overview:** After attending this presentation, attendees will gain knowledge concerning the relationship between low serotonin (5H-T) and aggression in children and adolescents. Attendees will understand that the burden of predicting violence and homicide risk assessment increasingly falls on the clinical psychiatrist.<sup>1</sup> This presentation will also discuss the potential of using serotonergic genetic markers to aid in the possible identification of aggression.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the genetic and biological relationship between low 5H-T and aggression in children and adolescents. Aggression is one of the most frequent concerns that trigger a child and adolescent psychiatric referral.<sup>2</sup> Youth homicides and assault-related injuries result in an estimated \$18.2 billion in combined medical and work-loss costs.<sup>3</sup> Studies have shown that low 5H-T has been associated with aggression in animal and adult studies. However, there has been a paucity of studies in relation to children and adolescents and the results have been inconsistent.

Aggressive behavior is complex and associated with multiple biological, psychological, and environmental factors. Aggression is often divided into two types: impulsive and premeditated. Neuromodulators associated with aggression include dopamine, norepinephrine, MAOA, and nitrous oxide synthetase. Serotonin remains one of the better-understood neuromodulators involved in impulsive aggression. A systematic literature search was conducted of the following databases: Embase®, PubMed®, PsycINFO®, and Google® Scholar. Studies published through January 25, 2018, were considered. The key words aggression, violence, adolescent, adolescents, juveniles, serotonin, 5-HT, 5-HIAA, 5-hydroxyindoleacetic acid, tryptophan, serotonin transporter gene polymorphism, 5-HTTLPR, and serotonin plasma membrane transport proteins were used, which included a PubMed® “Mesh” search. Inclusion criteria for the review included children and adolescents <18 years old, English language peer reviewed journals, physical aggression as the dependent variable, and serotonin as the independent variable. Exclusion criteria were the following: case reports, review articles, posters, abstracts, published in languages other than English, studies that defined aggression solely as suicide, greater than >18 years old, animals studies, and participants with co-morbid substance use disorders. One hundred fourteen studies were initially identified, and 12 studies were included for the review. The 12 studies can be grouped by how serotonin is measured into the following categories: cerebrospinal fluid (3), acute tryptophan depletion (1), prolactin response to fenfluramine (1) and genetic studies (7). The genetics articles reviewed included studies of the 5HT1B receptor polymorphism and the serotonin transporter gene (5-HTT or SLC6A4). Two common serotonin transporter gene polymorphisms include the 5-HTTLPR (promoter polymorphism), and the 5-HTT Variable Number Tandem Repeat (VNTR). Both are believed to regulate the transcription of the serotonin transporter gene.

The review found that the adolescent CSF studies had strong correlation with aggression. Studies of the 5-HTT (VNTR) and the 5HT1B polymorphism found no association with aggression. The rs16859448 TT Single Nucleotide Polymorphism (SNP) of the serotonergic FEV transcription factor had strong association with aggression. The studies looking at the 5-HTTLPR (promoter polymorphism), had mixed results but the strongest correlation in two gene X environmental studies that included other neuromodulators and environmental risk factors. Low childhood 5-HT function appears important but not sufficient for childhood aggression in the prolactin response to fenfluramine. Finally, in a study of Acute Tryptophan Depletion (ATD), the overall sample indicated increased aggression under the ATD. This presentation will also discuss behavioral and pharmacological treatments for youth aggression.

Limitations of the studies included relatively small sample sizes, adult controls in some studies, and primarily male and Caucasian samples. The use of broadband rating scales was often used rather than the more narrowband youth aggression rating scales. In conclusion, there appears to be a relationship to low 5-HT and aggression in children and adolescents. Further research is needed, particularly in the study of genetics and identifying potential future markers for identifying youth aggression.

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### Aggression, Serotonin, Adolescents



## I10 An Update on Female Arsonists: A Systematic Review

*Alick Wang, BSc\*, McMaster University, Hamilton, ON L8S4L8, CANADA; Yedishtra Naidoo, MD, McMaster University, Hamilton, ON, CANADA; Sebastien S. Prat, MD, St. Joseph's Healthcare, McMaster University, Hamilton, ON L8N 3K7, CANADA*

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**Learning Overview:** After attending this presentation, attendees will understand the recent developments in the understanding of the key psychopathologies and characteristics of female arsonists during this decade and explore the differences between previous research and more recent studies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting key aspects of the latest research in female arsonists and emphasizing the unique nature of female arsonists and the challenging aspects of understanding and treating them.

**Background:** The psychiatric literature regarding arson, fire setting, and pyromania is quite rich. However, despite key differences between male and female arsonists, there are relatively few studies directly investigating female arsonists or gender-related differences among fire setters. At present, there is only one review article on this topic, published in 2010. This study sought to perform a systematic review of the literature and determine whether there has been any evolution in the understanding of this topic since 2010.

**Methods:** Two independent reviewers conducted the literature search using the electronic databases MEDLINE®, Web of Science®, and PsycINFO®. The search was limited from January 1, 2010, to January 1, 2018. Study selection, data analysis, and reporting were conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Extracted data included the authors, year of publication, country of study population, study type, sample size, sample characteristics, gender, fire-setter status of the study population, age, definition of fire-setting behavior, and characteristics of female fire setters identified.

**Results:** A total of 270 articles were identified and seven were selected for review. Despite a large variation in methodology, including national surveys, case-control, and retrospective studies, most of the studies demonstrated consistent findings. Female arsonists are often victims of abuse, family disorganization, and low socioeconomic status. Studies published prior to 2010 describe depression and psychosis as key psychopathologies. More recent studies demonstrate a high proportion of substance abuse and personality disorders among female arsonists compared to male arsonists. There is also a higher prevalence of psychotic disorders when compared to non-fire setters.

**Conclusions:** Female arsonists are a unique patient population, distinct from both male arsonists and non-arsonist female offenders. This study reviews the existing literature and reveals that there has been some evolution in the understanding of female arsonists, although studies published before and after 2010 are mostly consistent. Hopefully, this knowledge will improve the understanding of female arsonists and help guide further research. Using the data collected from this systematic review, more detailed analyses could be performed in the future for a more detailed characterization of the literature.

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**Female, Arson, Systematic**





### I11 Spousal Homicide: What Do We Know and How Do We Prevent It From Happening?

Alexia Delbreil\*, *Chu La Milétrie Département De Médecine Légale, Poitiers, Nouvelle-Aquitaine 868021, FRANCE*; Jean-louis Senon, *University of Poitiers, Poitiers, FL, FRANCE*

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**Learning Overview:** After attending the presentation, attendees will: (1) understand the psychopathological basis of spousal homicide, (2) be able to identify the risk factors associated with this crime, and (3) understand how France and, more broadly, Europe, have developed prevention measures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing practical data of a crime that remains under-studied and by showing how to implement measures that are crucial for the safety of these victims.

Spousal homicide is a term used when the victim is killed by her/his partner or ex-partner. This type of murder is the most frequent one when looking at murders happening within the family circle. It also constitutes 16% to 20% of murders. Prevention measures regarding spousal homicide are under-developed because of being perceived as an exceptional situation. However, research has shown that the offenders committing spousal homicide have a well-defined profile, along with their *modus operandi*, compared to the other types of homicide.

The circumstances leading to such a tragic event are related to several factors, including the personality of each individual of the couple, situational crisis, and the quality of their relationship. This crime is often portrayed by the media as a horrific one, scaring the community, notably because it occurs in a close relationship, which is supposed to be where anyone could feel safe. This crime is primarily committed by males, aged 30 to 60 years old, when the couple is in the process of breaking up or when there is persistent conflict in their relationship. The home is where this crime commonly occurs. Alcohol is frequently found as a contributing factor at the time of the offense. Prior violent behavior of the offender toward the victim is also often a characteristic. Contrary to other types of homicide, no current or past psychiatric history is found. From a pathopsychological perspective, jealousy and willingness to control the victim are often seen as precipitating factors. Other psychological elements of the offender can explain why he decides to act out, such as anger, fear of abandonment, and the need to make these feeling stop. At this point, research regarding female offenders in the context of spousal homicide remains limited. So far, the data suggests that their motivation is different from male offenders. This speaks to the necessity of continuing studying this area to understand the origin of this behavior.

National and European measures were recently developed to help improve the support of women victims of violence from their partner and to find the most appropriate means to prevent any escalation toward lethal actions. These measures may not help resolve every situation because there remain cases in which no prior violence existed before the impulsive and lethal act of their partner. It is necessary to know the common risk factors that are associated with the spousal homicide situation in order to prevent it from happening. Informing professionals who may be in the best position to detect these situations, but also the public, is necessary to help these potential victims.

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#### Homicide, Domestic Violence, Acting Out



## I12 Linguistic Indicators for Psychological Violence Detection in the Language of Domestic Abusers

Victoria Guillén, PhD\*, University of Alicante, San Vicente del Raspeig, Alicante 03690, SPAIN

**Learning Overview:** After attending this presentation, attendees will understand language indicators for psychological violence detection in the family unit, as well as linguistic techniques and methods for that purpose.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how to detect psychological violence in cases involving domestic violence and how to gather the necessary evidence in language to demonstrate its presence.

Forensic linguistics-analyzed evidence is examined in this presentation for psychological violence detection in the language of domestic abusers. According to the Istanbul Convention (*The Council of Europe Convention on Preventing and Combating Violence against Women and Domestic Violence*) of May 11, 2011, domestic violence is: "... acts of physical, sexual, psychological, or economic violence that occur within the family or domestic unit or between former or current spouses or partners, whether or not the perpetrator shares or has shared the same residence with the victim." (Chapter I, Art. 3.b) Domestic violence is a surface manifestation of the power and dominance that some men exert over women in the family unit. It is often the case that when the victim tries to empower herself or break up with the offender, he will then try to regain power and control over her by using different strategies, ranging from psychological violence to force, whose purpose is to influence her decision and resume the relationship. Although in theory, the law protects women and children from the different forms of violence related to the social phenomenon of domestic violence, in practice, it is very difficult to prove psychological violence in court, for the very simple reason that, unlike physical and sexual violence, psychological violence leaves no ostensive trace in the target victim. However, its effects and consequences, as psychologists claim, can be even more devastating and destroying for the victim. The personalities of both the aggressor and the victim, as well as the effects of psychological violence on the psyche of the victim, have been studied extensively in the field of psychology. However, to the best of our knowledge, psychological violence has not yet received specifically dedicated scientific study in the field of forensic linguistics.

The goal of this presentation is twofold. On one hand, this presentation analyzes linguistic indicators that can be useful for psychological violence detection in cases involving domestic violence. On the other hand, this work presents the linguistic technique and methods that proved to be fruitful for the detection of psychological violence in such cases. These include semantics, speech act theory, discourse analysis, genre theory, and quantitative analysis. It is hypothesized that the language of the domestic abuser provides the linguist with direct access to different manifestations of psychological violence, (e.g., harassment, stalking, and emotional manipulation.)<sup>1,3</sup>

This presentation raises two research questions: Can linguistics help us detect indicators of psychological violence? If so, what type of evidence can the linguist collect in the language of the offender to show that there are indicators of psychological violence? The investigation is grounded in the empirical work conducted in a court case. The corpus of questioned documents consists of six letters and the abundant Whatsapp messages the offender sent to the target victim over 270 days.

Findings from this piece of empirical research show that psychological violence is inflicted by the domestic abuser over the target victim through malicious language use. Consequently, language is employed as a weapon against the target victim and "... acts as a precursor to and instigator of forms of conflict, and typically accompanies acts of violence in all its forms" (e.g., obsessional harassment, stalking, emotional manipulation).<sup>4</sup> Moreover, the offender typically resorts to strategic language use since it is designed to achieve a long-term or overall goal.

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### Forensic Linguistics, Psychological Violence, Emotional Manipulation



### I13 Bullying and Sexting on Social Networks

*Cinzia Gimelli, PsyD, PhD\*, Science & Method, Reggio Emilia 42124, ITALY; Luciano Garofano, PhD\*, Accademia Italiana di Scienze Forensi, Parma 43100, ITALY*

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**Learning Overview:** After attending this presentation, attendees will have an integrative viewpoint about this complex field of cyber crime from a psychological and design perspective.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing how availability and the use of social networking sites creates both opportunities and risks for young users.

This presentation evaluates the applicability of the current legal framework to cyberbullying and sexting, two types of (potentially harmful) behavior that are increasingly occurring between peers in the social networking environment. This analysis includes a mapping of applicable legislative provisions at the European and Italian level, an analysis of the Terms of Service of the largest social networking provider, Facebook®, as well as an overview and assessment of self-regulatory initiatives that have been taken by the industry in this area in Italy. The goal is to identify several solutions for a comprehensive strategy to ensure that risks of cyberbullying and sexting are dealt with in a manner that empowers young users.

**Introduction:** At young ages, there is an increase in reports of intimidation, harassment, intrusion, fear, and violence experienced through Information Technologies (IT). Hacking, spamming, identity theft, child pornography, cyberbullying, and cyber stalking are just a few examples of cyber crimes. In this study, the most common types of cyber crimes, epidemiological data, and the profiles of cyber victims and aggressors are approached. The studies that identify the factors contributing to IT misuse and to growing online vulnerability, principally in adolescents, are also discussed. Likewise, the central explanatory theories for the online victimization and the risk factors for victimization and perpetration online are addressed. Some cyber crime prevention strategies are anticipated, particularly among young people, seeking to provide clues to the consolidation of recent policies, namely at the digital design level.

The new age of mobile communication brought on by the internet has meant that people now have mobile access to a wealth of information and services. Although the benefits of mobile information access are acknowledged through the empowering influence over its audience, a concern is noted with reference to largely uncensored forums offering mobile communication exchange to young generations. The proliferation of mobile technologies available, in conjunction with applications facilitating social networking, has steadily increased the attack surface minors are exposed to in an online environment. Most minors engaging in online activities do so through mobile technologies such as cell phones. This device, because of its mobility, offers access to the internet that circumvents controls of supervision.

**Results:** This multidisciplinary study presents an approach that offers an alternative to existing solutions, available to the commercial market, that are driven by static configurations. This newer form of communication has many benefits to adolescents and plays an important role in their social lives. Despite the numerous benefits of these technologies, there are also several potential risks that can be detrimental to the mental health and well-being of adolescents.

**Conclusion:** This study proposes the necessity of performing revisions in clinical psychological practices and assessments, such as in forensic and legal policies regarding bullying and cyberbullying. In addition to improving legal success, this will reduce bullying prevalence rates, psychological distress, and psychopathology that can be comorbid or emerge because of this behavior.

This proposal will benefit young generations engaging in online interactions through the implementation of proactive strategies and it is critical that child psychologists, mental health providers, and forensic experts be informed of these potential risks so that effective forms of prevention can be found to educate adolescents and their families.

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**Public Health, Cyberbullying, Social Networks**



### I14 The Rorschach Test and Perpetrators of Homicide

*Ignazio Grattagliano, PsyD\*, University of Bari, Bari 70124, ITALY; Salvatore Zizolfi, MD, SSPsa Swiss Society of Psychoanalysis, SRR Roman S, Como 22100, ITALY; Antonella Valerio, Section of Criminology and Forensic Psychiatry, Bari 70110, ITALY; Daniele Zizolfi, MD, School of Specialization in Psychiatry, Faculty, Como 22100, ITALY; Stefania Zecca, PhD, Rorschach Psychotherapeutic Expert, Bari 70100, ITALY; Alessio Ostuni, MD, Sections of Legal Medicine and Criminology, Bari 70124, ITALY; Roberto Catanesi, MD, Bari 70124, ITALY*

**Learning Overview:** The goal of this presentation is to show that criminal activity—just as in any other human behavior—is a reflection of one’s personal history and level of psychic functioning.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that criminal activity, as with any other human behavior, is a reflection of one’s personal history and level of psychic functioning (i.e., organization of personality).

**Objective:** The hypothesis of this study is that it is possible to identify correlations between certain aspects of mental functioning in addition to criminological and forensic variables that are closely related to a crime. Moreover, this study hypothesized that a trace of this correlation exists in the Rorschach test.

The existence of a correlation between aspects of personality and crime has been theorized and that criminal activity reflects the personality traits of the perpetrator, his life, and the way he interacts with the world around him. Investigative psychology was born and developed on the assumption that behaviors expressed during an act of aggression, the choice of the victim, and the mode of action is, in some way, connected to the personal history of the aggressor and to his mental and behavioral functioning, in addition to the quality of his relationships.

**Methods:** This study examined the Rorschach protocols administered to a sample of homicide perpetrators who underwent psychiatric examination as ordered by Judicial Authorities to ascertain the ability of the subjects to understand their intent and will at the time the crime was committed. These data were taken from the database of the Section of Criminology and Forensic Psychiatry of the University of Bari, Italy, from 1998 to 2014. The goal of this study was to identify psychometric and psychodiagnostic variables associated with characteristics of the crime committed and correlate them to the crime scene, describing them as either “organized” or “disorganized.” The sample consisted of 49 subjects—43 males (87.7%), and 6 females (12.2%), 24 unmarried (48.9%), 21 married (42.9%), and 4 separated (8.2%). Education levels ranged from 0 years (5 subjects, 10.2%) to 13 years (9 subjects, 18.4%), while the majority had an education level of five years (15 cases, 30.6%), and eight years (20 cases, 40.8%). Twenty-four subjects were between 25 and 44 years old (48.9%), and 11 subjects were between 45 and 54 years old (22.4%). Eleven subjects were between 55 and 64 years old (22.4%); two of the subjects were minors, and one was older than 65 years of age. Eight of the subjects had no previous psychiatric diagnoses (16.3%). The following psychiatric diagnoses were formulated for the remaining 41 subjects (83.7%): 13 schizophrenia, 1 delusional disorder, 1 bipolar disorder, 5 depression, 4 psychoorganic syndrome, 13 personality disorder, and 4 limited intellectual functioning.

The results of this psychiatric report indicated that 23 (46.9%) subjects were recognized as fully chargeable, 10 subjects (20.4%) were shown to have a partial defect of mind, and 16 subjects (32.6%) were shown to have a total defect of mind. The means used to carry out the offenses are as follows: 15 (30.6%) used a sharp object or cutting weapon, 12 (24.5%) used a firearm, 8 (16.3%) resorted to physical assault, 6 (12.2%) used asphyxiation, and 8 (16.3%) through other various means (3 stoning, 3 precipitation, 2 by means of an automobile).

In 14 cases (28.6%), the murder was considered “premeditated.” In the remaining 35 (71.4%), the homicides were found to be “impromptu and not premeditated.” In 31 cases (63.3%), the crime scene was described as “organized.” In 16 cases (32.6%), the crime scene was described as “disorganized.”

**Results:** The Rorschach test has been confirmed as a particularly effective and sensitive tool in the study of normal and pathological personalities, including within the field of forensic psychiatry, when it is rigorously applied to standardized, reliable, and validated methods of administration, labeling, and evaluation, in addition to the use of representative samples of normal subjects. It is particularly evident that some of the Rorschach indices may be significantly correlated with the crime scenes that are described as “organized” and “disorganized.”

**Conclusion:** This study has confirmed how the Rorschach test, which is certainly useful in the clinical study of thought functioning, the examination of reality, affectivity, and of the ability to correctly represent oneself and others in relationships, can offer one psychic split that may also play another important role in the field of forensics.

#### Homicide, Perpetrators, Rorschach Test

### I15 Firearm-Bearing Rates During Neuropsychiatry Hospital Admission

*Oktay Cavus, MSc\**, Üsküdar University, Istiklal Cad No 108 Aznavur Pasaji Kat 5 Beyoglu, Istanbul 34430, TURKEY; *Gokben Sayar, PhD*, Uskudar University, Altunizade Mah Haluk Turksoy Sk No 14, Uskudar, Istanbul 34662, TURKEY; *Sevil Atasoy, PhD*, Uskudar Universitesi Adli Bilimler Enstitüsü, Altunizade Kampusu, Uskudar, Istanbul, TURKEY; *Kadri Dalgic, MAFS\**, Krav Magen Academy, Istiklal Cad No 108 Aznavur Pasaji Kat 5 Beyoglu, Istanbul 34330, TURKEY

**Learning Overview:** The goal of this presentation is to assess the demographic characteristics and psychiatric diagnostic profiles of subjects admitting to a psychiatric hospital while bearing a firearm.

**Impact on the Forensic Science Community:** This presentation will impact the forensic sciences community by drawing attention to the importance of securing a hospital environment and reducing the potential risks for violence and/or suicide.

Most completed suicides and homicides are reported to be committed with firearms. Although psychiatrists are in a strategic place to reduce firearm morbidity and mortality, the number of studies investigating the practices of firearm risk management is limited.

Data was pooled from the hospital databases to evaluate the admissions to a neuropsychiatry hospital with a firearm between January 2011 and December 2016. The medical files of the subjects who were reported to have a gun with them were evaluated.

Data revealed that 384 (0.0074%) of 51,666 subjects admitted to the hospital had a firearm. Forty-four individuals who were admitted to the hospital with a firearm were excluded due to readmissions, so 340 subjects were included in the study. A clear majority of the subjects who were admitted to the hospital with a firearm were males (96.7%,  $n=329$ ). The diagnosis of the subjects with a firearm were as follows: anxiety disorders 18.2% ( $n=62$ ), major depressive disorder 13.8% ( $n=47$ ), alcohol- and substance-related disorder 22.9% ( $n=78$ ), schizophrenia 7.6% ( $n=26$ ), bipolar disorder 8.5% ( $n=29$ ), and other disorders 3.5% ( $n=12$ ). Additionally, 25.2% ( $n=86$ ) of the subjects who were admitted with a firearm were recorded as being a visitor of an inpatient. Although 340 subjects were admitted to the hospital with a firearm, 254 of them were patients and only 157 (61.8%) of the patients were registered as a “gun owner” on the medical files. In the cases of the other 32.2%, the gun ownership status of the patient was not detected by the psychiatrist or psychologist.

It is concluded that during admission to the hospital, a careful security search for the presence of weapons is necessary. Electronic equipment such as metal detectors can assist in detecting firearms and securing the hospital environment. Considering the multitude of psychiatric patients carrying weapons, a more detailed psychiatric evaluation must be considered in gun license applications. Strategies must be developed for effective gun owning prevention without exacerbating stigma or discouraging psychiatric patients from seeking treatment.

#### Mental Illness, Firearm, Admission



### I16 I Am Not Pregnant: A Multidisciplinary Approach in a Case of Feticide Due to Denial of Pregnancy

*Ilaria Santoiemma\**, Bari 70124, ITALY; *Alessio Ostuni, MD*, Sections of Legal Medicine and Criminology, Bari 70124, ITALY; *Matteo Favia*, Bari, ITALY; *Sara Sablone, MD*, Institute of Legal Medicine, Bari 70124, ITALY; *Francesco Introna, MD*, Dim Sezione Di Medicina Legale, Bari 70124, ITALY; *Felice F. Carabellese, MD*, University of Bari, Bari 70124, ITALY; *Roberto Catanesi, MD*, Bari 70124, ITALY

**Learning Overview:** After attending this presentation, attendees will be aware of the complexity of a case of feticide, due to the particular prior relationship between the murderer and the victim.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of using a multidisciplinary approach, including psychiatric-criminological investigation, forensic-pathologist analyses, radiological imaging, and genetic testing, to provide the judge with a key to interpretation of the crime.

Feticide is a rare crime, with a percentage in Europe that ranges from 0.07% in Finland to 8.5% in Austria, per 100 births. It consists of the homicide of a newborn by its mother during delivery or afterward.

The presented case regards the death of an infant found on the seashore. After investigations were conducted by the police, a 22-year-old woman was charged with the murder of her own child.

Two inspections of the seashore were made. In the first, the baby was found supine, almost completely buried by the shore and the waves. The body was not rigid, hypostasis was not present, and the temperature of the corpse was uniform with the water's temperature. The umbilical cord was fragmented; no blood or sign of mummification were present. Reddish areas with an irregular distribution were present on the anterior part of the body. All the postmortem parameters used to estimate the time since death suggested four to five days. The second inspection was made a few days later because of the discovery of a dark stain on the seashore that turned out to be blood.

A DNA test was performed and showed a match between the baby and the 22-year-old woman and also confirmed the blood found on the stain belonged to both the baby and the mother. An X-ray, a Computed Tomography (CT), and a sonogram were performed and revealed a bi-parietal transverse fracture and regular pulmonary ventilation. The autopsy confirmed the cranial fracture and that no malformations were observed. All analyses concluded that the baby's death occurred a short period after delivery, and the cause of death was a hemorrhagic shock secondary to the non-completion of the umbilical cord.

Due to its complexity, the case required a multidisciplinary approach to establish the reasons behind the mother's unusual behavior. After a psychiatric evaluation, it was determined that the mother was affected by a borderline and narcissistic personality disorder, a low IQ with high level of egocentricity, and affective and cognitive immaturity. Nevertheless, this diagnosis was not enough to explain the crime committed. It was decided that the unusual defensive mechanism of the mother toward the gestation was denial of the pregnancy. From the psychiatric investigation, it also emerged that this was her second pregnancy and the first one also was characterized by denial behavior.

Denial of pregnancy is a rare condition and remains a phenomenon little known to health care professionals. It is characterized by a woman's lack of awareness of being pregnant; this is a dysfunctional defensive mechanism that does not allow the physical and emotional development in which women adapt to their future maternal role.

From a psychiatric point of view, denial of pregnancy can be divided into two groups: psychotic and non-psychotic. After the psychiatric investigation, it emerged that the denial of the pregnancy was a non-psychotic type and was based on a prolonged affective disorder, which means that the woman was not psychologically prepared to give birth. Based on the denial of pregnancy, feticide can be considered an impulsive, not predetermined, behavior fueled by a sense of anguish due to the sudden and unexpected confrontation of the denied reality—the birth. The mother's solution was the most primitive but functional—throw out the baby.

The described case poses challenging legal and psychiatric issues that require careful consideration. In the first place, the diagnosis of this type of disorder is important in Italian law because it can change the imputation, with a significant reduction in the number of years of sentencing in prison and, secondly, because it is important for the judge to have an explanation for such a dramatic behavior that is in contrast with social expectations before issuing a sentence.

#### **Feticide, Denial of Pregnancy, Personality Disorder**



### I17 Overkill by Proxy

*Alessio Ostuni, MD, Sections of Legal Medicine and Criminology, Bari 70124, ITALY; Giuliana D'anna, University of Bari-Section of Legal Medicine, Bari 70124, ITALY; Federica Mele, MD\*, Institute of Legal Medicine, Bari, ITALY; Davide Ferorelli, Bari, ITALY; Biagio Solarino, PhD, Università degli Studi di Bari, Bari 70125, ITALY; Roberto Catanesi, MD, Bari 70124, ITALY; Francesco Introna, MD, Dim Sezione Di Medicina Legale, Bari 70124, ITALY*

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**Learning Overview:** After attending this presentation, attendees will better understand an uncommon case of overkill by proxy and its significance from a forensic psychiatric standpoint rather than a forensic pathology standpoint.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the need for a universal definition of overkill and the importance of its possible variant from a criminalistic and juridical point of view.

Murder by proxy is a type of murder in which the murderer commits the act under the orders of another, acting as his or her proxy. There are two common scenarios for proxy murders: (1) a person takes a contract out on another person, and (2) a person manipulates a lover into murdering someone for him or her.

A 27-year-old man was found and extracted from a well. At first glance, a nautical rope tightened around the victim's neck and multiple traumatic injuries were observed. Autopsy findings and histopathological exams allowed the chronological reconstruction of events: a quarrel occurred inside an indoor sports complex causing contusions and defensive lesions. Subsequently, a nautical rope was tightened around the victim's neck. He was then dragged on the street and violently hit on the frontal region with a metal bar, causing skull fractures and brain hemorrhage. Last, a violent contusion with a large stone caused instant death because of a hinge fracture type 1 and the brainstem lesion. The murderer then tried to burn the victim's body, without results, then threw him into a well full of water, where the body was found.

The coexistence of multiple wounds and different modes of action led the forensic pathologist to classify this murder as overkill. A few hours later, the offender was arrested and the victim's partner (ex-wife of the killer) was also investigated. The man perpetrated the crime, but both people organized it, so a forensic psychiatric consultation was conducted on each of them.

The murderer admitted having killed his rival in love, incited by his ex-wife (who had shouted him, "If you want me, kill him."). The murderer's psychiatric evaluation documented an intellectual disability (IQ 41), a personality disorder, and a history of alcohol abuse. The ex-wife witnessed all the stages of the murder, without feeling any remorse toward her new partner. The psychiatric evaluation of the ex-wife documented a severe personality disorder (cluster B, with prevalence of narcissistic and borderline traits).

The crime story originates from a complicated relational linking, a "romantic" love triangle that involved the husband, the ex-wife, and the victim. The woman had a dominant personality, and easily manipulated her ex-husband; the man was a weak person, willing to do whatever to recover his marriage. She wanted to continue the romance with her lover, but when the victim wanted to stop the relationship, she decided to punish him ("If you are not mine, you will not be any other's."), planning the homicide using her ex-husband as a killer without getting her hands dirty.

The case presented is not a crime of impulse but a premeditated murder, fueled by revenge, committed in a rough and superficial manner.

Overkilling is considered a sex-motivated murder, with hatred and a destructive will toward the partner. However, what makes this case interesting is that the victim's partner was not the material author of the murder. So, it can be considered this is a real murder by proxy, but with typical methods of overkilling.

The collaboration between the forensic pathologist and forensic psychiatrist was very useful. The medicolegal analysis helped to direct the investigations toward a passionate crime; the psychiatric-forensic investigations allowed clarification of the dynamics of the murder and provided an explanation to such a cruel crime. The integrated work between forensic pathologist and forensic psychiatrist was fundamental to understand the meaning of such a brutal murder.

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### Personality Disorders, Overkill, Murder by Proxy



### **I18 “Corrective Rape” and Its Medicolegal Aspects**

*Anil Aggrawal, MD\*, Maulana Azad Medical College, Department of Forensic Medicine, Bahadur Shah Zafar Marg, New Delhi 110002, INDIA*

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**THIS ABSTRACT WAS NOT PRESENTED.**





## I19 The Psychopathologic Profile of Cannibals: A Review of Five Cases

Sophie Raymond, MD\*, Umd Henri Colin, Hôpital Paul Guiraud, Villejuif Cedex, Val De Marne 94806, FRANCE

**Learning Overview:** The goal of this presentation is to highlight the importance for forensic and health care practitioners to analyze the patterns of a crime scene, as well as the characteristics of both the offender and the victim.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing understanding of anthropophagy, by means of analyzing the cannibal's psychopathologic profile.

**Background:** In modern societies, human cannibalism (or anthropophagy) is rare and remains to most an unthinkable act of violence—a sensational crime that attracts the attention of the media. Classifications of cannibalism have varied over time. However, most authors agree on the existence of three categories, based on underlying motivations for this type of act: survival, ritual, and pathological. International psychiatric literature on the topic is sparse due to significant methodological problems, such as collecting enough data and generating unbiased analysis from adequately sized samples. The present work seeks to explore human cannibalism by presenting and comparing data concerning offenders, victims, and crime scene behaviors.

**Method:** This retrospective and descriptive study includes all five of the cannibal patients admitted to the Henri Colin High Secure Unit in France between 2000 and 2018. Information concerning sociodemographic, clinical, and forensic features was gathered from a variety of sources—medical records, police reports, written expert opinions, court transcripts, and autopsy reports.

**Results:** Most offenders were found to be men with a mean age of 26 years, who were mostly single, unemployed, living with the victim prior to the assault, and with a history of psychiatric disorder. The population of offenders falls into two psychopathologic profiles. First, three of them had a diagnosis of schizophrenia, with delusional themes of persecution and possession. The assault was characterized by brutality, lack of premeditation, precipitating factors (substance abuse and cessation of psychotropic medication). The act was part of a parricidal acting out, the ingested parts exclusively coming from relational zones (face, eye, phalanges). In the second group, the remaining two patients had a personality disorder with psychopathic and pervert tendencies. The act was part of a lethal acting out against a known victim and was characterized by the satisfaction derived from destruction, a desire for control, obsessions of omnipotence, and pre-existent cannibalism fantasies (paraphilia).

**Conclusion:** The results from the present study on five cannibal patients are consistent with case reports in the literature.<sup>1,2</sup> Offenders fall into two psychopathologic profiles: suffering from either a severe mental illness, such as schizophrenia; or a significant personality disorder comorbid with paraphilia. Several forensic features associated to each profile are detailed. Biases in this study do not allow for the generalization of these findings, and further studies are needed to better characterize this type of act.

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2. Pfäfflin F. (2008). Good enough to Eat. *Arch Sex Behav.* 37, 286-93.

**Cannibalism, Anthropophagy, Forensic Psychiatry**



### I20 The Vicarious and Literal Consumption of Identity: Interpreting Psychological Cannibalism

*Julian C.W. Boon, PhD\*, University of Leicester, Leicester, Leicestershire LE1 7RH, UNITED KINGDOM; Lynsey F. Gozna, PhD, University of Leicester, Leicester LE1 7RH, UNITED KINGDOM*

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**Learning Overview:** After attending this presentation, attendees will better understand psychological cannibalism and its putative etiology. Attendees will have also been advised on the ramifications for forensic intervention and law enforcement's effective case management stratagem.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) enhancing competence in identifying signs of psychological cannibalism, particularly in the context of stalking and threat cases; and (2) increasing the understanding and consideration of appropriately tailored assessment tools for utilization in investigative domains and in the context of therapeutic (forensic mental health) and correctional settings.

This presentation articulates the nature of a little-understood and previously unidentified form of cannibalism that is principally psychosexual in nature. This presentation states how psychological cannibalism represents an extreme form of stalking that could result in a heightened risk of reputational, familial, or physical harm.

Moving away from the physical act of cannibalism, it is argued that a certain psychological disposition can result in the quest for the identity of another, that is, someone for whom the perpetrator wishes to become. The underlying etiology of this psychological and corresponding behavioral presentation will be discussed in its various forms and include illustrations of cases. These briefly include some of the legion impersonators of the late celebrity Elvis Presley, the murderer of the Beatle John Lennon, and other non-celebrity examples.

The psychological challenges occur, it is argued, during the process and realization of the perceived transition from the rejection of "own self," followed by the search, identification, and fixation processes in the acquisition/development of the "new self." Cases have incorporated a focus on achieving a pseudo-celebrity, pseudo-intellect, and/or pseudo gender and sexual identity change. It will be argued that this can be an extremely tortuous and dangerous condition from a victim/investigative/treatment perspective. The pattern is not uncommonly that of adulation of an obsessional nature, which in time subsumes the victim's identity, lifestyle, and personal life. The consequences of this insidious process are not only painful for the primary victim, but also for their loved ones.

The cases range from the seemingly benign Elvis Presley pretenders to the lethal consummation of another's identity by causing their death. It will be discussed, physically consuming victims by psychological cannibalists is not a necessity, though they may wish to retain their body as reifying, comforting evidence of their having taken over the victim's identity. However, it is a possibility that when the fixated cannibal wants complete consumption/annihilation of another's fixated identity, only physical consumption would serve as being sufficient. This will be discussed in the context of a typology of cannibalistic need to set the scene for broader considerations of the challenges in risk and the prediction of future harm. Ultimately, the difficulties facing practitioners in understanding the presentation of psychological cannibalism are varied in regard to the complexities of harm.

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#### **Cannibalism, Extreme, Stalking**



## **I21 Serial Killers Seen on the Other Side of the Atlantic**

*Nidal Nabhan Abou, MD\*, Saint Baudelle, Mayenne 53100, FRANCE*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### I22 Child Pornography and Child Sex Dolls: An Expanding Virtual Phenomenon

Dean Michael De Crisce, MD\*, *Special Treatment Unit, Avenel, NJ 07001*; Sebastien S. Prat, MD\*, *St. Joseph's Healthcare, McMaster University, Hamilton, ON L8N 3K7, CANADA*; Eleanor B. Vo, MD\*, *OmaDesala Psychiatric Services, Ewing, NJ 08618-2111*; Jessica Morel, DO\*, *Fayetteville, NC 28306*; Renee Sorrentino, MD\*, *Institute for Sexual Wellness, Weymouth, MA 02188*; Matthew E. Hirschtritt, MD, *University of California, San Francisco, CA 94122*

**Learning Overview:** After attending this presentation, attendees will understand the global expansion of child pornography and other simulated and digital materials geared toward individuals with pedophilic arousal. Issues to be reviewed include: (1) international prevalence, (2) ease of access, (3) general internet phenomenon regarding child pornography, (4) simulated child pornography, (5) child sexual dolls, (6) characteristics of users, and (7) challenges in risk considerations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by elucidating the scope and expansion of materials geared to pedophilic arousal in order to assist forensic mental health experts in evaluating these behaviors.

Global expansion of online access has led to increased production, distribution, and use of digital child pornography. Where these materials were once difficult to obtain and store in print, they are now readily, and covertly, accessible in digital format through anonymous file sharing, on the deep web, and even on regular internet sites. Evidence gathered from identified child pornography users likely represents only a small fraction of the total number of individuals who access digital child pornography, as apprehension is limited. Further, little is known about users of simulated child pornography, child anime, and other such materials. Creators of these materials have attempted to avoid prosecution and appeal to users by using offshore servers, creating “simulated” child pornography, and even by manufacturing anatomical child “dolls” for sexual use. The scope of distribution and use of these items is staggering, and associated websites are estimated to have millions of viewers.

Evidence from forensic populations suggests that individuals who engage in digital child pornography, in contrast to those who commit hands-on child molestation, are more likely to be younger, to have completed college, to be employed, to be of higher socio-economic class, and less likely to have an active or history of a substance use disorder, serious mental illness, or significant antisocial traits. In addition, they are less likely than hands-on offenders to have a criminal history (e.g., incarceration or court appearance). However, like hands-on offenders, they are almost exclusively male. Though there is considerable debate in the field, there is mounting evidence that degree of engagement in digital child pornography (e.g., sorting and organizing image files, editing images, renaming files) is a stronger indicator of subsequent child pornography engagement, in part because of the ease with which large databases of pornography (that may unexpectedly include images of children) can be downloaded from the internet. Even less is known about child anatomical doll users, given the covert nature of their use. However, self-report data among adult anatomical doll users suggests that the clear majority are male, middle-aged (mid-30s to mid-50s), White, not partnered, well educated, heterosexual, and use their dolls for sexual stimulation and gratification.<sup>1</sup> Most anatomical doll users report revealing their use in online forums.

As the result of rapidly and continually expanding literature on sexual deviancy, the field has incorporated factors that have been empirically demonstrated to either contribute to, or protect from, sexual offense risk. Risk assessment methods have typically focused on hands-on offenders and utilize actuarial risk instruments that operationalize historical and dynamic risk factors to provide the most accurate risk prediction that has been available thus far. The field is moving toward greater standardization and heightened responsiveness to emerging literature, thereby enhancing the aims and integrity of forensic science. However, few instruments have been devised to assist in the assessment of solely child pornography users and none for digital or simulated child pornography users. As the phenomenon expands, this is an area of increasing concern and needed research.

This panel presentation will explore the online phenomenon of materials geared to pedophilic arousal, discuss known characteristics of users, and consider risk approaches to assessment when comparing hands-on offenders with non-hands-on offenders. Time for discussion will be available, including the ethics and legality of the availability of these materials.

#### Reference(s):

- <sup>1</sup>. *The Modern Sex Doll-Owner: A Descriptive Analysis*. Thesis presented to the Faculty of California State Polytechnic University, San Luis Obispo In Partial Fulfillment of the Requirements for the Degree Master of Science in Psychology by Sarah Valverde (formerly Schewe). August 2012.

#### Child Pornography, Pedophilic Disorder, Forensic Psychiatry



### I23 A Psycho-Criminological Profile of Child Sex Offenders

*Mathieu Lacambre, MD\*, French Forensic Psychiatry Society, 505 Rue De La Mogère, Montpellier, Languedoc-Roussillon 34000, FRANCE; Cindy Prudhomme, CHU, Avenue Doyen Gaston Giraud, Montpellier, FRANCE; Helena Berthet, CHU, Avenue Doyen Gaston Giraud, Montpellier, FRANCE; Philippe Courtet, MD, CHU, 371 Venue Doyen Gaston Giraud, Hôpital Lapeyronie, Montpellier, FRANCE*

**Learning Overview:** The goal of this presentation is to specify the psycho-criminological profile of perpetrators of sexual violence against minors and their respective dangers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving understanding of child sexual assault and prevention of sexual abuse.

Based on a cross-sectional, observational case-control pilot study of the neuropsychological and criminological characteristics of convicted child sex offenders, this study explored and compared several variables to specify neuropsychological alterations and their relationship to the aggression. The results of the evaluations of 64 men convicted of child sexual assault were compared to the results of 64 males (control) matched by sex, age, and level of education. The usual psychiatric criteria were reported (antecedents, current diagnosis and lifetime, treatment), and the following parameters were assessed: level of depression via the Montgomery–Åsberg Depression Rating Scale (MADRS), decision-making strategies via the Iowa Gambling Task (IGT), impulsivity via the Barratt Impulsiveness Scale (BIS 11), inhibition (the Stroop test), working memory (N-back), cognitive distortions (the Bumby scale), social desirability (the Marlow Crowne scale), and level of insight via the Beck Cognitive Insight Scale (BCIS).

Criminologically, according to data, homosexual acts are more often conducted outside the family circle; whereas, when they occur within the family, they will be repeated more often.

The main statistical analyses highlighted five distinct profiles: (1) Impulsive deficiency—low IQ, poor inhibition, high level of impulsivity in all three dimensions (cognitive, motor, and planning), history of depressive disorders and violent suicidal behavior, prepubertal victim(s); (2) Incestuous stepfather—rather old, high social desirability, alcohol-smoking with intoxication at times of aggression, insight based on certainties with low introspection, low impulsivity scores, pubescent victim(s); (3) Pedophile—elderly, without a psychiatric history, without taking drugs during the aggressions, low impulsivity, capacity of introspection, extrafamilial victims, homosexual; (4) Opportunistic—rather young, married, without children, without addiction, negator, already convicted for sexual offending, smoker, assault with violence of pubescent victim(s); and (5) Predator—left-handed, low level of insight, assault with violence, lack of denial, prepubertal and heterosexual victim.

These results clearly show a polarization between perpetrators of sexual violence suffering from current or past psychiatric disorders, several times assaulting a prepubescent victim without violence in the family, versus violent sexual aggressors assaulting pubescent victims with violence outside the family.

In addition, these data account for the particular neuropsychological alterations of the perpetrators of pedosexual aggression, but especially of the heterogeneity of the profiles. The precise evaluation of these dimensions should allow the evolution of specific interventions by prioritizing specific objectives (depression, addiction, insight, cognitive distortions, impulsivity) for targeted treatment (anti-impulse treatment, libido control, cognitive remediation) in an integrative (psychological, educational, psychiatric) manner.

According to the observed deficits, the coordination of targeted interventions would increase the effectiveness of the treatment of the patient on the one hand, and also actively work toward the prevention of the recidivism of the perpetrators of sexual violence, on the other hand, according to the profile met.

### Sex Offenders, Pedophilia, Risk Assessment



### I24 A Sample of Men Convicted of or Charged With a Sex Offense: Penile Plethysmography (PPG) Results

Emily D. Gottfried, PhD\*, Medical University of South Carolina, Charleston, SC 29407; R. Gregg Dwyer, MD, EdD, Medical University of South Carolina, Community & Public Safety Psychiatry Division, Charleston, SC 29407

**Learning Overview:** The objective of this presentation includes informing those involved in the assessment of persons who have sexually offended of considerations of the use of PPG as it relates to diagnosis and, in turn, risk of reoffending

**Impact on the Forensic Science Community:** The goal of this presentation is to discuss the data of a sample of men who underwent PPG testing to help standardize the assessment and add to the literature base of the use of PPG in this population.

The use of physiological measures in evaluations of sexual offenders has mixed reviews.<sup>1,3</sup> PPG has been called the gold standard of physiologically assessing male sexual arousal. It can be used to aid in the diagnosis of paraphilic disorders by identifying paraphilic sexual arousal. Although it has been shown to be valid and reliable in some studies, the use of the PPG in evaluations of sexual offenders has also been the subject of criticism<sup>1-4</sup> There has been a call for more research on the use of PPG in assessing men convicted or charged with a sex offense.

This presentation will focus on results from PPGs administered to a sample of approximately 80 men undergoing sexual behavior evaluations. The sample included men referred for a pretrial evaluation, assessment of sexual violence risk under a state's Sexually Violent Predator (SVP) Act, and professional sexual boundary violations. Many of the evaluations were assessing dangerousness for civil commitment under the SVP Act, which requires a prior arrest for a sexually violent offense and the presence of a mental abnormality or personality disorder that makes the examinee likely to engage in acts of future sexual violence if not civilly committed in a secure setting for long-term care and treatment. These evaluations took place in the Sexual Behavior Clinic and Lab of a large academic medical center in the southeastern United States from 2011 to 2018. Data will be presented on the number of PPG tests that did not meet minimum requirements for interpretation and the likely reasons as to why these tests were invalid, including rates of suspected dissimulation. Additionally, this presentation will describe the PPG scenarios that generated the highest level of sexual arousal for the whole sample and in men who only offended against children. Differences in level of responding between men with only child victims and those with pubescent/adult victims will also be discussed.

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**Sexual Offenders, Penile Plethysmography (PPG), Sexually Violent Predators**



## I25 Understanding Sexual Offenders: The Importance of Personality Dimensions

Ingrid Bertsch, MA\*, University Hospital Center of Tours, Tours 37044 Cedex 9, FRANCE; Christian Réveillère, Université De Tours, Tours, FRANCE; Thierry H. Pham, Mons, BELGIUM; Robert Courtois, PhD, Tours, FRANCE

**Learning Overview:** The goals of this presentation are: (1) to provide attendees with an understanding of how sexual offenders behave, (2) to learn how to describe the personality traits of sexual offenders, and (3) to be better prepared to apprehend sexual offenders through a global approach.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing further knowledge about: (1) violence and sexual violence, (2) understanding and evaluating a sexual offender's personality, and (3) the therapeutic approaches for sexual offenders.

The interest in sexual violence, and especially in the perpetrators, has intensified in recent years. The psychological heterogeneity of sexual offenders remains a major difficulty for therapists and researchers involved in understanding these patients.<sup>2</sup>

Among the factors of sexual offenders described in the literature, personality has been put forward as a factor of vulnerability or protection because it offers a holistic approach (impact on emotional feeling, behavior, and social relationships) while addressing the specific needs of everyone.

This population can be portrayed by studying the personality of sexual offenders from a clinical angle (using standardized clinical scales) and in relationship to normal functioning using the Big Five Inventory (BFI) model or bio-psycho-social models such as Temperament and Character Inventory (TCI).<sup>5-7,10,13</sup>

Compared to non-sexually violent patients, the personality of sexual offenders is marked by: (1) a negative emotional experience—they show a lack of self-confidence, loss of self-esteem and less narcissism, more intense feelings of negative emotions, an increase in the neurotic dimension, and will be more prone to develop mood disorders, rapid mood swings, and depression; (2) strong impact on social skills—they show signs of social stress, a decrease in extraversion and openness, difficulty maintaining appropriate social distance, more avoidant, dependent, passive-aggressive personalities, insensitivity to social cues, lack of cooperation, lack of self-direction, quirks, and the presence of schizoid or paranoid behavior; (3) impact on behavior—unlike non-sexually violent men, sexual offenders show better self-control, low impulsivity, drug addiction, presence of obsessive traits, less use of excess violence during assaults, and less sadism or anti-sociality.<sup>1-4,8-10,12,14,15</sup> They exhibit novelty-seeking that can lead to substance abuse.<sup>15</sup>

This study will be presented in two forms: a literature review dealing with the personality of sexual offenders and a presentation of the results of a preliminary study conducted with 30 French sexual offenders and 30 non-sexually violent offenders, assessed with the BFI.<sup>11</sup> The goal is to provide a global picture of the personality dimensions of sexual offenders, specific profiles of subgroups of sexual offenders (extra- or intra-familial, abusers of adults or children, hands-on or hands-off offenders), explanatory perspectives, and implications for therapy.

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### Personality, Sexual Offenders, Violence

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### I26 Sex Offender Reading Levels: Recidivism, Referral, and Age of First Offense

Taylor J. Willis\*, Claflin University, Orangeburg, SC 29115; Emily D. Gottfried, PhD, Medical University of South Carolina, Charleston, SC 29407; R. Gregg Dwyer, MD, EdD, Medical University of South Carolina, Charleston, SC 29407

**Learning Overview:** The goals of this presentation are: (1) to inform those involved in the assessment of sexual offenders of the considerations of reading levels as they relate to etiology and, in turn, the risk of reoffending, and (2) to provide treatment providers with data on the potential impact of reading ability for treatment planning use.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the ability of evaluators of sexual offending behavior to determine etiology and risk and by helping treatment providers develop treatment plans with a likelihood of successful outcomes.

This presentation focuses on the reading levels of 1,135 adults who committed sex offenses and were being reviewed for potential civil commitment under the South Carolina Sexually Violent Predator (SVP) Act. Previous studies suggest that the majority of inmates in America are functionally illiterate and that the improvement of basic skills would help decrease the rate of recidivism.<sup>1</sup> Other qualitative studies have reported a correlation between recidivism of juvenile delinquents and low academic achievement. These studies also reported a trend of below grade-level reading scores among incarcerated youth.<sup>2,3</sup> It was hypothesized that juvenile offenders who had been arrested and remanded to juvenile justice facilities would have lower reading levels than juvenile offenders who were not remanded to a juvenile justice facility. This study hypothesized that the reading level would increase as the age of the offender's first sex offense increased (i.e., individuals who had their first sex offense arrest at an older age would have higher reading scores than individuals who were of younger age at first sex offense arrest). It was predicted that the reading levels of offenders would decrease as the number of prior sexual offenses increased. It was also hypothesized that sex offenders who were referred to the next step in the process for civil commitment under the SVP Act would have lower reading scores.

Preliminary data indicated that reading levels ranged from 0 to 15.0 (15<sup>th</sup> grade) with an average level of 8<sup>th</sup> grade. Approximately 46% ( $n=520$ ) of the overall sample had a history of juvenile arrests for any offense, with 27.5% (129) of those inmates having been detained in a youth detention facility. In this sample, 31.9% of offenders had been convicted of a previous sex offense. Also, 42.2% ( $n=1,047$ ) of the offenders were referred to move on to the next step of being considered for civil commitment.

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#### Sexual Offenders, Literacy, Sexually Violent Predators





## I27 Characteristics of a Forensic Inpatient Sample in a Strict Security Facility: An Update

Natalie Armstrong Hoskowitz, PhD\*, Bridgewater, MA; Lauren Miller, PhD\*, Bridgewater State Hospital, Bridgewater, MA 02325; Joseph Toomey, PhD\*, William James College, Newton, MA 02459

**Learning Overview:** The goals of this presentation are: (1) to update attendees and add to the literature regarding descriptive characteristics of this understudied subpopulation, (2) to identify the frequency and type of referrals for forensic services and the appropriateness of these referrals, and (3) to make suggestions on how to address inappropriate referrals at the corrections and community levels.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by expanding the knowledge of forensic inpatient characteristics and inappropriate referral questions and applying this information to their own community, correctional, or inpatient settings for comparison.

Available research cataloging characteristics of individuals receiving inpatient psychiatric services have attended to systemic factors affecting their rate of admission for civil commitment (e.g., access to mental health care, socioeconomic status), as well as clinical and demographic characteristics of the patients within the hospitals that treat them (e.g., most frequent psychiatric diagnoses, length of admission, etc.).<sup>1,2</sup> However, less is known about the characteristics of individuals admitted for forensic inpatient services (e.g., treatment for competency restoration, criminal responsibility, etc.). Regarding these characteristics, much of the extant literature focuses on inpatient samples outside of the United States.<sup>2-5</sup> Currently, studies from the United States suggest individuals receiving forensic inpatient services may differ both within this group (e.g., cognitive impairment impacting competency restoration) and also from other inpatients (i.e., those involuntarily civilly committed) with regard to demographic and clinical characteristics (e.g., intellectual disability diagnosis).<sup>6-8</sup> Finally, the reasons that individuals are referred for forensic psychiatric services within the United States vary not only by reason (e.g., acute psychosis, behavior management, etc.), but also by location (e.g., referrals from the community, correctional settings, etc.). As a result, it is currently unknown how many of these individuals referred for services are appropriate for treatment in a strict security inpatient environment. Recently, there has been a call for a census of individuals populating forensic mental health inpatient facilities, and there is recognition in the psychiatric community that the future of mental health law will be at least partially predicated on understanding the characteristics of the population served.<sup>9,10</sup> This study aims to add to the literature by enumerating on the demographic and clinical characteristics of an updated sample of United States forensic inpatients in a strict security facility, as well as by identifying common referral reasons and the appropriateness of these referrals.

Data were obtained from an archival review of records of male inpatients from a strict security New England state hospital. Data will be obtained from July 2018 through January 2019. Demographic and clinical characteristics for inclusion are derived from studies cited in footnotes. Reasons for referrals (e.g., competency restoration, self-injurious behavior) will be also culled from the archival records. Referral appropriateness will be operationalized by comparing initial referral questions to the opinions in reports completed by forensic evaluators following the inpatient evaluation period. Specifically, the “goodness of fit” between evaluator opinions and the initial referral question will be coded qualitatively by forensic evaluators who did not author reports in archival records. Data will be analyzed using non-parametric and descriptive analyses in SPSS software. Results will be discussed in the context of practical applications for attendees, including suggestions on how to address inappropriate forensic referrals to inpatient facilities. Limitations and future directions will be presented.

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### **Inpatient, Characteristics, Referrals**



### I28 Implementing Cognitive Behavioral Therapy for Psychosis (CBTp) With Forensic Patients: The Identification of Barriers to Positive Treatment Outcomes

*Kyrsten M. Grimes, MA\*, University of Toronto Scarborough, 1265 Military Trail, Scarborough, ON M1C1A4, CANADA; Konstantine K. Zakzanis, PhD, University of Toronto Scarborough, 1265 Military Trail, Scarborough, ON M1C1A4, CANADA*

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**Learning Overview:** The goals of this presentation are: (1) to review the literature on the effectiveness of CBTp in forensic populations relative to non-forensic populations; and (2) to review the ways in which forensic patients with schizophrenia differ from their non-forensic counterparts, particularly with respect to factors that may act as barriers to positive treatment outcomes.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating clinicians on factors that are likely to impact treatment outcomes when implementing CBTp. It will also serve to advance research on utilizing CBTp with forensic patients by qualitatively synthesizing the literature.

It is generally established that patients with schizophrenia have impairments in both neurocognition and metacognition. It is posited that deficits in neurocognition may place an individual at greater risk of developing deficits in metacognition. According to cognitive theories of psychosis, hallucinations and delusions develop, in part, because of metacognitive deficits. Recent research has begun to target these deficits in psychosocial treatments, such as CBTp. CBTp attempts to reduce positive symptoms by targeting the underlying cognitive biases thought to underlie them. Research generally indicates that CBTp is effective in reducing positive symptomatology, with medium- to large-effect sizes. However, very little research has investigated the efficacy of CBTp in forensic samples. Research in forensic populations is often undertaken with the purpose of identifying risk factors for violence. Research examining in what ways forensic and non-forensic patients with schizophrenia differ in any other regard, particularly with respect to neurocognition and metacognition, is lacking. The efficacy of psychosocial interventions for psychosis in forensic samples should be examined independently of non-forensic samples, as forensic patients often have numerous comorbidities, which may influence the severity of metacognitive and neurocognitive deficits and therefore impact treatment effectiveness.

Of the research that has been conducted, the findings generally indicate that CBTp is less effective for this group than non-forensic patients with schizophrenia with little overall change. The implication of the following factors on metacognition will be reviewed as it pertains to forensic populations: comorbid personality disorders, history of violence, and substance use. Taken together, each of these factors independently are associated with deficits in metacognition, either directly or indirectly through associated deficits in neurocognition, though greater research is needed in these areas. As such, it is hypothesized that forensic patients with schizophrenia may represent a subgroup of patients that are more resistant to CBTp due to greater impairment in metacognition.

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#### CBTp, Schizophrenia, Treatment Outcomes



## I29 The Relationship Between Mental Illness, Criminal Offenses, and Discharge

Simmi Patel, BS\*, Voorhees, NJ 08043; Serge Sevy, MD, Manhattan Psychiatric Center, New York City, NY

**Learning Overview:** After attending this presentation, attendees will understand the difficulty in discharging patients who have a history of incarceration and psychiatric illness. During this presentation, the relationship between mental illness, criminal offenses, and discharge will be discussed as well as the issues related to the legal and mental illness system of discharging patients with criminal offenses who suffer from psychiatric disorders.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by educating attendees on the complex issues of discharging a stable psychiatric patient with a criminal history.

Due to the stigma and potential consequences of discharging a psychiatrically stable forensic patient, Loch argues that discharging a patient back to the community is a daunting and complex task that can be unfavorable to many patients.<sup>1</sup> Fazel et al conducted a study that involved 12,056 patients of which 53% were violent offenders.<sup>2</sup> That study concluded that there is evidence from psychiatric services that forensic patients have a lower offending rate than other groups being discharged. The study argues there is reluctance by hospital teams to discharge patients due to the “problematic transition” from the hospital to the community. The stigma and lack of assistance for forensic patients impedes their discharge into the community.<sup>2</sup> For many forensic patients, after prolonged hospitalization, there is a need of assistance and close supervision. The goal of the discharge objectives is to assist the patient as well as protect the public. Due to the potential concern of forensic patients committing a crime in the community due to psychiatric illnesses, the issue that arises is: the boundary between the right of the patient to be discharged versus the right of the public to be protected.<sup>3,4</sup> Violent behavior, social maladjustment, and stigma are negative setbacks that play an important role in discharge for forensic patients. This case report will illustrate the challenges of discharging a stable psychiatric patient who committed a serious crime due to a psychiatric illness.

The discussion will present the case of a 54-year-old African American female with a complex history of criminal offenses and a diagnosis of schizophrenia. The patient has multiple incidents of self-injuries secondary to auditory hallucinations. She was incarcerated from June 1982 to June 1987 and given a three-year probation sentence for armed robbery. She was later charged with murder and served 22/25 years for first-degree manslaughter. However, at the time of her release, she was transferred to a state psychiatric facility due to non-compliance and similar delusions to her instant offenses. Due to her poor response to anti-psychotics, she was treated with clozapine, which was titrated to reach therapeutic level. The patient improved significantly; however, she continued to suffer from paranoid, grandiose, and religious delusions. The patient has failed three Human Forensic Committee (HFC) interviews, which has brought her discharge into a rather complex situation. At her first hearing in September 2017, she was not approved for discharge because of her persisting delusions and intent to purchase a gun after discharge. Consequently, she failed another HFC interview in October 2017 because she believed that her crime was staged and that the man she killed was brought back to life. She believed that she was acting in good faith. Subsequently, through several interviews from November 2017 to June 2018, the patient has denied her intent to buy a gun. She believes that a gun is “inappropriate and improper.” Currently, the patient feels comfortable and confident enough to call the police if she ever feels threatened in the community. Due to the therapeutic effect of clozapine, she is more rational and logical, despite some persistent delusions. She is pleasant on the wards and participates in group activities and discussions. Her team feels ready to discharge her and the patient is awaiting another HFC interview.

This presentation will discuss the question: how is discharge affected by persisting psychiatric symptoms for patients who have a criminal history? This presentation will provide information from psychiatric evaluations, psychological evaluations, human forensic committee results, past medical history, criminal history, and previous psychiatric hospitalization.

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### Forensic Patient, Incarceration, Discharge



### I30 A Poorly Thought-Out Method to Reduce Homelessness: Proposed California Assembly Bill 1971

Aadhar R. Dhamecha, MD\*, Redondo Beach, CA 90277

**Learning Overview:** The goals of this presentation are: (1) to summarize California Assembly Bill (AB) 1971 that expands the definition of grave disability for involuntary civil commitment, (2) to review the primary purposes of this legislation with regard to decreasing homelessness in California, (3) to review the evidence and opinions for and against AB 1971, and (4) to discuss the proposed bill's potential legal and societal ramifications on the community and forensic psychiatry as well as on persons who are homeless and mentally ill.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a thorough analysis of AB 1971. The bill's medical, psychiatric, legal, and public ramifications will be illustrated through a case example.

According to existing California civil commitment laws under the Lanterman-Petris-Short (LPS) Act, if a person, as a result of a mental illness, is a danger to others, a danger to self, or gravely disabled, he or she may be taken into custody and placed involuntarily in a facility for 72-hour treatment and evaluation.<sup>1</sup> Currently, "grave disability" means a condition in which a person, as a result of a mental illness or chronic alcoholism, is unable to provide for his or her basic personal needs for food, clothing, or shelter.<sup>1</sup>

AB 1971 expands the definition of "grave disability" to include a condition in which a person, as a result of a mental illness, is unable to provide for his or her basic personal needs for medical treatment, if the failure to receive medical treatment results in a deteriorating physical condition that will more likely than not lead to death within six months.<sup>1</sup>

Proponents of the bill cite that more than 800 individuals who were homeless died on the streets of Los Angeles County in 2017.<sup>2</sup> They suggest that the bill would prevent such unnecessary deaths in those who are both homeless and unable to tend to their medical needs because of their mental illness. Consequently, the bill would allow such individuals to be civilly committed and be given an LPS conservator who would act as a surrogate medical decision-maker in order for the conservatees to obtain necessary treatment for their medical conditions.<sup>2</sup>

Opponents of the bill argue that there are no data that demonstrate how many of those who were homeless and died suffered from mental illness that impaired their willingness to seek medical care.<sup>2</sup> Opponents also cite a lack of current or planned infrastructure to treat and house the population this bill is meant to help, further questioning the effectiveness of the bill in its stated purpose.<sup>2</sup>

Legal issues include whether this bill infringes upon the civil rights of those who are homeless and mentally ill. Opponents argue it is dangerously expansive at the expense of individual liberties.<sup>2</sup> Additionally, the passage of this bill would require multiple physicians, rather than one, to address the specific criteria of "grave disability." That is, psychiatrists would need to assess symptoms of mental illness and whether they impact the patient's capacity to refuse medical care, and other medical specialists would need to assess the diagnosis, treatment, and prognosis of the patient's medical condition. Moreover, the fiscal impact is expected to be significantly large with increased medical, legal, and housing costs that, according to analysts, are unpredictable and non-reimbursable, a gloomy outlook for a state that already has a high cost-burden in public services.<sup>2</sup>

Scrutinized in its entirety, AB 1971 brings more problems than solutions in its attempt to combat the homelessness crisis in California.

#### Reference(s):

1. California Assembly Bill 1971, 2017-2018, Regular Session (California 2018), [http://leginfo.legislature.ca.gov/faces/billNavClient.xhtml?bill\\_id=201720180AB1971](http://leginfo.legislature.ca.gov/faces/billNavClient.xhtml?bill_id=201720180AB1971).
2. California Assembly Floor Third Reading Analysis, California Assembly Bill 1971 (California, May 2018), [http://leginfo.legislature.ca.gov/faces/billAnalysisClient.xhtml?bill\\_id=201720180AB1971#](http://leginfo.legislature.ca.gov/faces/billAnalysisClient.xhtml?bill_id=201720180AB1971#).

#### Civil Commitment, Homelessness, Refusal of Medical Care



### I31 **Assessing the Propensity for the Future Risk of Revenge in Secure Forensic Settings and in the Community: An Evidence-Based Approach**

*Lynsey F. Gozna, PhD\*, University of Leicester, School of Psychology, George Davies Centre, Leicester LE1 7RH, UNITED KINGDOM*

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**Learning Overview:** The goal of this presentation is to develop awareness of an approach to the holistic assessment of the risk of harm regarding revenge-oriented thoughts, fantasies, and acts. This will be particularly relevant for forensic practitioners from criminal investigation to prosecutors and broader forensic mental health and correctional settings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by heightening understanding of the complexities and inter-relationships between revenge-oriented personalities, underlying mental health conditions, cognitive processes, and, ultimately, behaviors intended to cause harm. This approach seeks to assist the consideration of how to best tailor the choice/administration of psychometric assessments while increasing confidence in the creation of revenge-based formulation from the most recent evidence-based review.

It is proposed that there is a critical need for forensic practitioners to develop a consideration of revenge when working with clients in applied settings, not only in regard of recent offenses, but in a holistic manner to ensure that risk is fully conceptualized. Although revenge is considered routinely as one of the more prevalent motives in the context of criminal justice and clinical forensic practice, its conceptualization requires more in-depth interpretation to truly formulate risk of future harm and the propensity for revenge-oriented acts. The definitional challenges further require revenge to be assessed more fully than at a surface-level (proximate) understanding of the original transgression and the response, resulting in the potential for limited psychological insights. Ultimately, the act of revenge is the outcome of several complex processes requiring consideration of any underlying predisposition and the baseline propensity for revenge (trait and state), the relevance of any prior traumatic events experienced, cognitive processes such as fantasy and violent ideation, grievance thinking and rumination, and the resources and opportunities available to act.

Currently, the possibilities for the assessment of revenge are challenging in that any approach is heavily focused on the emotion of anger with limited consideration of broader elements that can stimulate such behavior and impede the capacity to develop a holistic interpretation and risk formulation. This is critical when attempting to identify the roots and routes of revenge and how this can be fueled in complex, dynamic environments, whether already brewing or through the exploitation of vulnerable individuals for whom revenge can become a new narrative and focus. This presentation will outline challenges in comprehensively assessing propensity for revenge to ensure a psychologically meaningful outcome.

A psychometric critique of measures comprising anger and aggression, personality (normative and disordered), post-traumatic stress and embitterment, empathy, rumination, violent ideation, forgiveness, and vengeance/revenge will be presented. This has been based on a consideration of revenge across offenses and contexts and further draws on findings from a recent scoping review on human-induced trauma in addition to discussions with subject matter experts in the fields of forensic mental health and trauma. Hence, it has been possible to develop a process model for considering the critical elements of revenge when conducting tailored formulations of risk.

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#### **Revenge, Risk, Assessment**



### I32 Ethical Challenges in Forensic Psychiatric Practice

*Joseph Ferencz, MD\*, St. Joseph's Healthcare, Hamilton, ON L8P 3K7, CANADA*

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**Learning Overview:** The goal of this presentation is to provide an overview of the challenges associated with the practice of psychiatry in a forensic context with specific attention given to the conflicts associated with multiple simultaneous roles and their potential resolution.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by identifying and clarifying potential ethically challenging situations in the practice of forensic psychiatry, and by posing alternative potential solutions to be considered.

The discipline of psychiatry poses ethical issues that are unique in medical practice. The authority to detain, the reliance on subjective assessment, poorly defined boundaries as related to roles, diagnoses, and treatments all contribute to frequent ethical conflicts, morally distressing decisions, and the potential for abuses. Furthermore, the nature of the relationship between psychiatrists and their patients may be intensely personal and patients may be particularly vulnerable. For reasons such as these, it has been suggested that psychiatry requires its own unique approach to ethical problem solving. Forensic psychiatry, as a subspecialty, brings with it a variety of additional challenges that have been explored by a number of commentators. The ethical risks associated with issues of dual agency, confidentiality, bias, and expert competency are ever present. Consequently, forensic psychiatric practitioners must be particularly vigilant and familiar with their professional codes of conduct. The practice of recovery-oriented care with individuals who have been found unfit to stand trial or not criminally responsible, and who are subject to dispositions of the courts or review boards, presents an additional array of ethical tensions. The assessment and management of risk in a rehabilitative context raises many issues related to autonomy, confidentiality, and conflicting roles.

This presentation will focus on several of the complex ethical scenarios that arise in the treatment and rehabilitation of forensic psychiatric patients and will discuss several current approaches to their analysis. The issues associated with the provision of clinical services, such diagnosis, acute treatment and rehabilitative care, and risk-related activities such as dangerousness assessment and risk management, will be explored. A number of proposed solutions to these dilemmas will be reviewed with regard to their strengths and weaknesses. A specific integrative model for the management of these conflicts will be presented.

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**Forensic, Psychiatry, Ethics**



### I33 Mistaken Identity: Cultural, Religious, and Ideological Beliefs in Forensic Evaluation

Sheresa Christopher, PhD\*, Medical University of South Carolina, Charleston, SC 29407; Emily K. Shier, LPC, NCC, Medical University of South Carolina, Charleston, SC 20407

**Learning Overview:** The goal of this presentation is to educate attendees about various religious, cultural, and ideological beliefs that have the potential to be misidentified by forensic evaluators as being psychological in nature. This presentation also seeks to help evaluators recognize these beliefs in their practice and discuss implications for forensic examinees and the legal system.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving understanding of the ideas or concepts held by subgroups of the population that may be mistaken for psychosis in forensic evaluations. Appropriate identification of these beliefs will in turn increase the accuracy of diagnosis in a forensic context and thus provide more accurate and valid information for the referring court system.

This presentation will focus on cultural and religious considerations in forensic evaluation. Further, this presentation will discuss ideas or concepts held by sub-groups of the population that may be mistaken for psychosis.<sup>1,2</sup> Although it is clear that culture, religion, and ideology must be considered in clinical and forensic evaluation, several cultural, religious, and ideological beliefs may be mistaken for symptoms of mental illness (e.g., delusional beliefs or perceptual disturbances).<sup>3</sup>

For instance, individuals who identify as sovereign citizens believe the United States government does not have authority over them.<sup>4</sup> They express beliefs in a Uniform Commercial Code, Admiralty Court, note copyrights are placed on names, and discuss having beliefs related to individuals who identify as a corporation. Further, they derive meaning from the use of capital letters when writing names, cite the importance of the Fourteenth Amendment, and speak of Redemption. Similarly, individuals who identify as Christian may describe experiences in which God is communicating with them and speaking audibly at times.<sup>1</sup> They may assert that God is responsible for providing them with direction, consolation, and empowerment, and/or reference the existence of prophecy. While the above-described beliefs may be accurately identified as delusional at times, they are not innately pathological in and of themselves and require a knowledgeable and skilled evaluator with understanding of cultural and religious considerations in the context of forensic assessment.

Mistaken identification of these beliefs as psychotic in nature may have a significant negative impact on individuals who hold these beliefs as well as on the legal system. This presentation will briefly outline several different cultural and religious beliefs and discuss the ways in which they may be falsely identified as psychotic in nature. Specifically, beliefs held by those who identify, practice, or believe in ideology who identify as Christian, Muslim, Sovereign Citizens, Moorish-Americans, Voodoo, Freemasons, those who believe in reptilians, or those who hold culturally based attitudes regarding trust. Additionally, the implications of misidentifying symptoms of mental illness for legally involved individuals and the legal system as a whole will be discussed.

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2. Pytyck, J., Chaimowitz, G.A. The Sovereign Citizen Movement and Fitness to Stand Trial. *International Journal of Forensic Mental Health*, 2013, 12, 149-153.
3. Shepherd, S., Lewis-Fernandez, R. Forensic Risk Assessment and Cultural Diversity: Contemporary Challenges and Future Directions. *Psychology, Public Policy, and Law*, 2016, 22 (4), 427-438.
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#### Cultural Considerations, Religious Considerations, Forensic Evaluation





### I34 The Phenomenon of Suicide-Pesticide: Case Reports and a Review of the Literature

Federica Fersini\*, Viale Felsina 16/2, Bologna, ITALY; Chiara Palazzo, MD, Via Imerio 49, Bologna, ITALY; Paolo Fais, PhD, Department of Medical and Surgical Sciences, Unit, via Imerio 49, Bologna, ITALY; Susi Pelotti, MD, University of Bologna, Paolo Fabbri 88, Bologna 40100, ITALY

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**Learning Overview:** After attending this presentation, attendees will consider the phenomenon of suicide-pesticide and the importance of a comprehensive forensic investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by confirming that companion animals had significantly greater odds of being a medicolegal case in all categories.<sup>1</sup>

The combined event of a suicide and the killing of a pet is an event relatively unexplored in forensic literature. Frequently, the killing method is the same for human and pet, but, according to Oxley, little is known about how the bond between humans and pets may infer suicidal and/or homicidal ideation.<sup>2,3</sup> Forensic psychiatrists have tried to study this phenomenon considering the significance of the killing of a pet, as the animal is part of the family. In some individuals, the bond with pets prevents them from committing suicide.

In fact, concerns about who would care for their pets after they commit suicide could potentially influence the ideation of killing the pet before or together with suicide. Certainly, postmortem examination and forensic analysis could reveal key elements to better characterize similar events.

Recently, the phenomenon in the United States and the United Kingdom was investigated through media reports showing the variety of circumstances related to extended suicide. A number of similar cases have been experienced in Italy, but not all cases come to the attention of the forensic pathologist, so the phenomenon is underestimated.

Here is reported two case studies: one is a planned complex suicide-“pesticide” of a woman and her dog perpetrated by the ingestion of psychoactive drugs in combination with self-strangulation of the woman and intentional drug fatal intoxication in the dog; the second is a “pesticide”-homicide-suicide casework.

Data obtained from the death scene investigation, autopsy, and toxicological analyses will be reported.

#### Reference(s):

1. McEwen B.J. Trends in Domestic Animal Medico-Legal Pathology Cases Submitted to a Veterinary Diagnostic Laboratory 1998–2010. *J Forensic Sci.* 57, no. 5 (September 2012): 1231-233.
2. Cooke B.K. Extended Suicide With a Pet. *J Am Acad Psychiatry Law.* 41, no. 3 (2013): 437-43.
3. Oxley J.A., Montrose V.T., Feldman M.D. Pets and Human Suicide. *J Am Vet Med Assoc.* 249, no. 7 (October 2016): 740-41.

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#### Suicide-Pesticide, Autopsy, Death Scene Investigation



### I35 A Fatal Pulmonary Embolism in a Hospitalized Psychiatric Patient: A Medicolegal Analysis

Biagio Solarino, PhD, Università degli Studi di Bari, Sezione di Medicina Legale, P.zza Giulio Cesare, 11, Bari 70125, ITALY; Alessio Ostuni, MD\*, Sections of Legal Medicine and Criminology, Policlinico of Bari Italy, Piazza Giulio Cesare 11, Bari 70124, ITALY; Alessandro Dell'Erba, PhD, Risk Management Unit, Policlinico Teaching Hospital of Bari, P.zza Giulio Cesare, 11, Bari 70124, ITALY; Salvatore Moliterno, MD, Section of Legal Medicine University of Bari, P.zza Giulio Cesare 11, Bari 70124, ITALY; Roberto Catanesi, MD, P.zza Giulio Cesare, Bari 70124, ITALY

**Learning Overview:** After attending this presentation, attendees will understand that venous thrombosis among patients with severe mental disorders can result from various mechanisms that cause blood vessel injury or venous stasis, potentially determining fatal consequences due to the occurrence of a Pulmonary Embolism (PE).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by deepening awareness about specific psychiatric disorders and how guidelines must be incorporated in order to prevent fatal thromboembolic complications and to, ultimately, avoid the risk of a lawsuit for alleged negligent medical care.

Psychiatric patient mortality is three-fold higher than the rate in the general population and nearly 60% is due to cardiovascular disorders. Specific risk factors included lifestyle issues and adverse effects of long-term medication, as well as an increased occurrence of obesity, diabetes, and hypertension.

Venous Thromboembolism (VTE) is the combination of PE and Deep Venous Thrombosis (DVT) and is known as a risk factor for sudden death in patients hospitalized in mental health units.

PE accounted for nearly 4% of unexpected sudden deaths in psychiatric patients; nevertheless, risk factors of VTE have not systematically been investigated in psychiatric inpatients.<sup>1</sup>

The main causative factor in these cases is immobility associated with venous stasis. Injuries to the extremities during physical restraint or compression and a reduction in blood flow of the extremity veins may also have prothrombotic effects enhancing tissue factor expression in endothelial cells, resulting in thrombosis formation.

Treatment with antipsychotics, especially phenothiazines, results in an increased platelet aggregation. Metabolic symptoms caused by antipsychotics, such as body weight increase, hyperleptinemia, hyperglycemia, and dyslipidemia, are known to be risk factors for VTE and to interfere with the coagulation and fibrinolysis processes.

**Case:** The case of a 45-year-old man with bipolar affective disorder and metabolic syndrome was reported. He went to the Psychiatric Emergency Department for management of a psychotic mood disorder associated with violence and property damage. During hospitalization, he was difficult to manage and aggressive toward the medical staff. Hence, he was given clonidine and lorazepam, then treated with aripiprazole and valproic acid. Four-point and two-point physical restraints were intermittently applied for five days. The vital parameters were normal, apart from occasional tachycardia. After removal of the physical restraints three days later, he collapsed and all efforts to resuscitate him failed. The patient's family filed a wrongful death lawsuit against the hospital, alleging that negligent medical care at the psychiatric ward led to his death. A medicolegal examination was requested.

An external examination of the body revealed obesity (1.85 m; 120 kg) in the absence of traumatic injuries. At autopsy, a saddle embolus completely occluding the right and left pulmonary arteries and extending throughout the peripheral branches of the pulmonary arterial circulation was observed. Dissection of the deep veins showed patchy occlusive thrombosis of the left popliteal vein, extending the ipsilateral saphenous vein. Morphological and histological heart and kidney examinations were in accordance with hypertrophic cardiomyopathy and renal chronic disease. PE was identified as the cause of death.

**Discussion:** A comprehensive analysis of the patient's medical records was performed. During hospitalization, the patient had never experienced cardiovascular or pulmonary symptoms. The patient's obesity, age, and immobility assessed low or intermediate clinical probabilities for pulmonary thromboembolism. Hence, the possible medical liability for not having administered heparin prophylaxis was herein discussed.

**Conclusion:** The goal of this case is to underline the importance of making a correct analysis of VTE risk factors in hospitalized psychiatric patients. This case further suggests the need to incorporate the presence of psychiatric disorders and exposure to antidepressants and/or antipsychotics in scoring systems for pulmonary thromboembolism.

#### Reference(s):

1. Jiří Masopust, Radovan Malý, Martin Vališ. Risk of Venous Thromboembolism During Treatment With Antipsychotic Agents. *Psychiatry and Clinical Neurosciences* 2012;66:541–552.

#### Venous Thrombosis, Mental Disorders, Negligent Medical Care



### I36 Awareness and Attitudes of Health Care Professionals in Unperceived Pregnancies

*Oktaý Cavus, MSc, Üsküdar University, İstanbul 34430, TURKEY; İnci Y. Tezbasan, BSc\*, Uskudar University, İstanbul 34662, TURKEY; Gokben Sayar, PhD, Uskudar University, İstanbul 34662, TURKEY; Sevil Atasoy, PhD, Uskudar Üniversitesi Adli Bilimler Enstitüsü, İstanbul, TURKEY*

**Learning Overview:** The goal of this presentation is to determine the level of awareness and attitudes of health care workers regarding unperceived pregnancies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing the importance of better training regarding pregnancy for first responders, health care professionals, and teenagers.

Unperceived pregnancy is a phenomenon in which the expectant mother is unaware of her pregnancy despite its advanced stage. The expectant mother becomes aware of her pregnancy either at 20-weeks gestation or later. While the identification of the pregnancy at 20-weeks gestation is defined as “partially unperceived pregnancy,” the identification of the pregnancy just before the birth or during labor is defined as “total unperceived pregnancy.”

This study sought to determine the level of awareness and attitudes of health care workers regarding unperceived pregnancies. A sociodemographic form and awareness and attitude questionnaire were used as data collection tools in the research. In this questionnaire prepared by the researchers, two clinical vignettes were given and the participants’ views about the table were asked after each clinical vignette. The study group consisted of 240 people with a mean age of 31.3 years and a standard deviation of 8.3. When the participants were examined according to their occupation, it was observed that 71.2% were psychologists, 16.2% were psychiatrists, and 12.5% belonged to other health services. The results of this study suggest that the psychologists, psychiatrists, and other health workers who participated in the research were indecisive to the vignettes that define unperceived pregnancies and that they had difficulty in recognizing and interpreting the clinical situation.

Thinking that the clinical situation may be associated with concealed pregnancy, psychosis, or mental retardation due to a clinical mental disorder, not knowing pregnancy signs and that the situation may be explained by inexperience, that it may be due to negligence and irresponsibility, that it may be the unconscious denial of an unwanted pregnancy, or that the woman was not aware of her reproductive capacity were all considered in close proximity. The fact that one-fourth to one-third of the participants were indecisive for each question shows the confusion the table created. The most important step to take for the early detection of unperceived pregnancies, in order for the mother candidate to have appropriate pregnancy follow-up and psychological support, for the birth and growth of the newborn to be physically and psychological healthy, and in order to prevent the forensic problems and familial crises that unperceived pregnancies would create, is to raise awareness about the unperceived pregnancies for, first, the health care personnel and then for society.

#### Unperceived Pregnancy, Health Care Professionals, Awareness



### I37 Sudden Death Caused by Hyponatremia Related to Psychogenic Polydipsia

Rohan P. Kedar, MD\*, Department of Psychiatry, Livonia, MI; Joseph A. Prahlow, MD, Western Michigan University School of Medicine, Kalamazoo, MI 49007

**Learning Overview:** After attending this presentation, attendees will understand that sudden death may occur as a result of Psychogenic Polydipsia (PPD), a condition associated with various psychiatric conditions, especially schizophrenia.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting two cases of sudden death occurring in schizophrenic patients, wherein autopsy findings disclosed abnormal vitreous electrolyte values consistent with low-salt/hyponatremia produced by PPD.

Sudden, unexpected death in the context of psychiatric illness is not rare. Common scenarios include suicides and accidental deaths related to substance abuse. Other scenarios include deaths related to concurrent medical conditions, such as heart disease, deaths from trauma, and deaths related to complications of therapy.

PPD represents a disorder characterized by the compulsive consumption of large amounts of water as a secondary effect of an underlying psychiatric illness. Importantly, there is no intent of self-harm, and there is no identifiable underlying organic explanation for the behavior, such as a hormonal imbalance. Patients with schizophrenia are at most risk for PPD. In severe cases, dilutional hyponatremia can occur, with subsequent brain edema, seizures, coma, and death. This report presents two cases of sudden unexpected death occurring in schizophrenic patients in which autopsy findings determined that the cause of death was related to PPD.

Case 1 involved a 48-year-old, mentally handicapped, schizophrenic man who was noted to be in distress while at his group home. Emergency Medical Services (EMS) personnel were summoned and found the man in asystole. All resuscitative efforts were to no avail. In addition to schizophrenia, the man suffered from grand mal epilepsy, renal insufficiency due to lithium damage, with a known medical history of PPD, for which he had been placed on fluid restriction. A medicolegal autopsy was performed, which disclosed a slightly enlarged and dilated heart, mild coronary artery atherosclerosis, but no other gross abnormalities. Microscopic examination and toxicology testing were non-contributory. Vitreous electrolyte measurement revealed a sodium of 78mEq/L (normal: 135mEq/L–155mEq/L) and a chloride of 58mEq/L (normal: 105mEq/L–135mEq/L). The cause of death was ruled as: part I–seizure disorder; part II–PPD due to schizophrenia. The manner of death was considered natural.

Case 2 was that of a 47-year-old man who was found dead in his bed. He had a known past medical history of schizophrenia and obesity. A medicolegal autopsy was performed, at which he was noted to have marked cardiomegaly (heart weight of 640 grams; normal <400 grams), as well as mild coronary artery atherosclerosis. Microscopic examination confirmed the myocardial hypertrophy but disclosed no other significant findings. Urine and drug screens were negative for drugs of abuse. Vitreous electrolyte testing revealed a sodium level of 85mEq/L and a chloride level of 110mEq/L. The cause of death was ruled as: part I–hyponatremia due to PPD related to schizophrenia; part II–cardiomegaly and atherosclerotic cardiovascular disease. The manner of death was natural.

Although PPD is most common in schizophrenic patients, it may also affect patients with other psychiatric disorders, including mood disorders and substance use disorder.<sup>1</sup> The exact mechanisms underlying the development of PPD are not known. However, psychiatric medications are not generally considered to contribute significantly, as there are known cases of PPD occurring prior to the use of currently prescribed psychotropic medications.<sup>2</sup> Patients with PPD are at increased risk of morbidity and mortality. The cases presented serve to remind the medical and psychiatric communities that PPD is a potentially lethal manifestation of psychiatric illness.

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2. deLeon J., Verghese C., Tracy J.I., Josiassen R.C., Simpson G.M. Polydipsia and Water Intoxication in Psychiatric Patients: A Review of the Epidemiological Literature. *Biol Psychiatry.* 1994;35:408-19.

#### Psychogenic Polydipsia, Hyponatremia, Sudden Death



### I38 The Brains of Suicides by Violent Methods Reveal a Distinct Biology

*Giovanna Punzi\**, Baltimore, MD 21231; *Gianluca Ursini, MD, PhD*, Baltimore, MD 21205; *Leonardo Collado Torres, PhD*, Lieber Institute for Brain Development, Baltimore, MD 21205; *Joo Heon Shin, PhD*, Baltimore, MD 21205; *Roberto Catanese, MD*, P.za Giulio Cesare, Bari 70124, ITALY; *Andrew E. Jaffe, PhD*, Baltimore, MD 21205; *Thomas M. Hyde, MD, PhD*, Baltimore, MD 21205; *Joel E. Kleinman, MD, PhD*, Baltimore, MD 21205; *Daniel R. Weinberger, MD*, Baltimore, MD 21205

**Learning Overview:** After attending this presentation, attendees will understand how the distinct state of mind associated with suicide by violent means may relate to a more salient biology that can be observed postmortem in brain tissue.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a novel key to suicide biology derived by behavior analysis.

Suicide is the tenth leading cause of death for all age groups combined and is on the rise across the United States, according to recent reports by the Centers for Disease Control and Prevention (CDC). As suicidal ideation is a limited predictor of outcome, recent research has turned to the detection of “more objective” biological markers (i.e., genetic marks) for the development of prevention approaches. This may be especially relevant for suicides by violent means, since features of impulsive aggression represent a better predictor of self-destructive acting out, a strong behavioral endophenotype, as well as a valuable research target, being associated with higher prevalence and lethality. In this regard, in completed suicides, the biology underlying the choice of a violent method should represent a more precise feature to target to detect genetic signatures for the behavior at large. Of note, in searching for suicide biomarkers, most studies do not address the relevance of the means adopted, hence they do not consider the possibility of a method-specific frame of mind that ultimately generates the behavior.

Previous discovery findings and further replication suggest that differences in Dorso-Later Prefrontal Cortex (DLPFC) expression of a human-specific non-coding RNA (lincRNA) may influence emotional regulation, aggressive behavior, and suicide by violent means. In the present study, RNA-sequencing (RNA-seq) data from postmortem human brain (228 Caucasian patients; adults) were examined to validate, at a genome-wide level of significance, association of the lincRNA specifically with suicide by violent means and to detect further candidates potentially related to the same signal.

Attribution of suicidal method was determined blind to the postmortem RNA-seq data. Cause and manner of death and contributory causes or medical conditions related to death were obtained from medical examiner documents. The choice of a specific method likely reflects the interplay of multiple determinants, including the availability of a particular suicidal means. However, at the individual level, preferences toward one or the other group appear to influence the ultimate pattern of choices. Cases in which manner of death was pending or not determined at the time of the curation and suicidal samples with ambiguous, or indefinable means of suicide, regarding the level of violence employed, were excluded. Among the remaining suicides, most deaths distinctly fell within the violent or non-violent category. When this was not obvious, an in-depth behavioral assessment was obtained using detailed narrative summaries based on all available sources of historical information, including interviews with next of kin. The differential expression analysis was conducted on all features, including genes, exons, junctions, and expressed regions data, correcting for diagnosis, sex, age, and qSVs, a measure of RNA integrity. A gene-set enrichment analysis was also performed on the top-list differentially expressed features. At  $P_{FDR-corr.} \leq 0.05$ , minimal signal (i.e., ~10 expressed regions) arose when comparing non-suicide with suicide (all types of method); differentially expressed features further decreased when looking only at suicide by non-violent means. However, comparison between non-suicides and suicides specifically by violent means produced a remarkably greater list of features (i.e., over 1,400 expressed regions). These results validate, at a genome-wide level of significance, previous findings and suggest the engagement of specific signaling and cellular type. These results confirm that classifying suicide by method is key in revealing the underlying different biology.

#### Brain, Suicide, RNA-Seq

### 139 The Contribution of Suicide Notes in the Resolution of Doubtful Cases of Suicide: From the Psychological Autopsy to the Forensic Evidence—A Case Report and Review of the Literature

Isabella Aquila, MD\*, Institute of Legal Medicine, University Magna Graecia of Catanzaro, S Venuta-Medicina Legale, viale Europa, Catanzaro 88100, ITALY; Ada Maida, MD, Institute of Legal Medicine, Viale Europa, 88100, Catanzaro, ITALY; Santo Gratteri, MD, Viale Europa, Germaneto, Catanzaro 88100, ITALY; Fabrizio Cordasco, MD\*, Università Magna Graecia CZ, Viale Europa, 88100, Catanzaro, ITALY; Luigi De Aloe, MD\*, Institute of Legal Medicine, Viale Europa, 88100, Catanzaro, ITALY; Roberto Raffaele, BE\*, University Magna Graecia of Catanzaro, Via Thailandia N 1, Crotona, ITALY; Valerio Riccardo Aquila\*, Via Dante Alighieri, Crotona, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Section of Legal Medicine, University of Foggia, Foggia 71100, ITALY

**Learning Overview:** After attending this presentation, attendees will understand the contribution of the suicide note in the resolution of doubtful cases of suicide.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by underlining the role of the psychological autopsy in suicide cases.

Suicide is a public health issue. Among young people, it represents the second cause of mortality. Approximately 10,000,000 people die every year from suicide and this number will rise to 15,000,000 by 2020.<sup>1</sup>

Italian epidemiological studies report that hanging (52.1%) is prevalent among men; falling (35.1%) and hanging (33.4%) is prevalent for women. A major propensity for suicide is found among older people.<sup>2</sup> Suicide is related to psychiatric disorders, previous attempts of suicide, alcohol or other substance abuse, a history of mistreatment, divorces, and unemployment.

Often, a suicide note is present, which is a letter left by the victim to explain the reasons for the suicide. From these notes, we can analyze the cause, the contest, and the choice of suicidal method. It is crucial proof in the investigation because it helps us understand, in doubtful cases, if it is an actual suicide. However, a suicide note can be also falsified.<sup>3</sup>

There are cases in which the suicide note is not present. In these cases, it is possible to perform a so-called psychological autopsy, a retrospective investigation into the victim's life and the potential causes that led to the suicide. The method consists of thorough structured interviews of relatives, friends, or doctors, collecting all available information about the victim to obtain a valuation of his/her life, personality, mental health, and relationships.<sup>4</sup>

There are different types of suicide notes (i.e., farewell, instructions, accusations of others, requests for forgiveness, and justifications), and they are often handwritten on paper. This facilitates, through a calligraphic comparison, the demonstration that the victim, and no other, wrote the note, rejecting the hypothesis of murder.<sup>3</sup> There are some cases in which the victim writes the note on clothes or on his/her skin, while with the advent of new technologies, young people also use email, social networks, and telephone messages. In these cases, the so-called social-mobile autopsy is conducted.<sup>5</sup>

Reported here is the case of a middle-aged woman found dead at home. The interview of relatives by the police highlighted that the woman's son found her body hanging from a rope knotted to an internal railing of the dwelling. However, during the scene inspection, the forensic pathologist and the investigators found the woman's body lying on a bed, with the rope cut at the node. The external examination of the woman presented a double-cutaneous furrow with characteristics of vitality and bruising. The morphology of the furrow insinuated doubts about the mode of death considering the contrasting witnesses of family members and the movement of the body, which did not allow understanding of exactly how it was suspended. During the investigation of her house, investigators found, in the bedroom, a handkerchief with the following suicide note: *Prayer before falling asleep to Mary. O Virgo, it's late. Everything falls asleep on the earth, it's time for rest, do not move away from me. Put your hand on my eyes like a good mother, close them to the things below. My soul is full of troubles and sadness the fatigue that awaits me is here near me. Put your hand on my forehead and stop my thought, sweet will be my rest if blessed by you because tomorrow the poor son wakes up stronger and happily resumes the weight of the new day. Put your hand on my heart and give back to God an eternal love. Amen*

The site inspection is very important in discriminating the circumstances in doubtful cases, in finding incongruities, background, and motivation that allows an explanation of the facts. The forensic investigation does not end with the detection of signs and analysis of the suicide location, but there is a clear necessity of coordination between the various sources of information: relatives, friends, and acquaintances, as well as diaries, notebooks, notes, computers, and more. The psychological autopsy is a new method to better understand the circumstantial data of the crime scene investigation, postmortem examination, and autopsy.

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#### Forensic Sciences, Suicide, Psychological Autopsy

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## I40 When Freedom of Speech Harms: 13 Reasons Why and Its Influence on Suicide in Youths

Sharon L. Guo, MD\*, USC Institute of Psychiatry and Law, Los Angeles, CA 90033

**Learning Overview:** After attending this presentation, attendees will: (1) know the difference between protected and prohibited speech as guaranteed by the First Amendment, (2) become familiar with the landmark cases relating to the First Amendment and the media, and (3) have a better awareness of how past legal decisions may change because of technological advances in media.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising their understanding of the phenomenon of suicide contagion; in particular, how media can influence vulnerable youth to model self-injurious behavior. In addition, there will be a discussion of landmark cases in which the media's freedom of speech was challenged based on its negative impact on society, namely, inciting violence.

Evidence of the effect of the media on suicide has been supported by the literature. The "Werther effect" was coined in 1974 by sociologist, David Phillips, after a rise in suicides committed by young men after reading Goethe's 1774 novel, *The Sorrows of Young Werther*, in which the tragic protagonist ends his life. Phillips substantiated the phenomena of copycat suicides, which has been supported by many ensuing studies. Another important consideration is age-specific effects because youth tend to imitate behavior, such as that shown in suicide clusters. Therefore, when the media focuses on suicide, youth may be highly susceptible to engaging in suicidal behavior.<sup>1-5</sup>

In 2017, Netflix®, a media service self-described as "the world's leading internet television network with 130 million subscribers in over 190 countries," released the show, *13 Reasons Why*.<sup>6</sup> In the season finale, the 16-year-old protagonist is shown cutting her wrists with a razor and then bleeds out and dies in a bathtub.<sup>7</sup> In the weeks and months following the program's debut, at least one Los Angeles County psychiatric emergency department noticed a surge of young patients who were admitted involuntarily. For example, a mother brought her adolescent daughter to the emergency room after the daughter sent her a suicidal text message with a knife emoji the morning after viewing *13 Reasons Why*.

Considering the content in *13 Reasons Why*, which depicts suicide and targets a teenage audience, the constitutional protection of freedom of speech under the First Amendment may be challenged. The United States Supreme Court case in *Brandenburg v. Ohio* held that freedom of speech is not protected when it is "directed to inciting or producing imminent lawless action and is likely to incite or produce such action."<sup>8</sup> The use of this reasoning in subsequent legal cases whereby the media's right to freedom of speech was upheld, as well as cases in which the media were censored and found liable, will be presented.

This presentation will not only discuss the impact of *13 Reasons Why* on the viewing public but will also highlight whether there may be a need to rethink the First Amendment guidelines in the 21<sup>st</sup> century. In addition, how the right of the media's freedom of speech can be balanced with that of society's right to be protected from harm, via its application in blogging, internet privacy, and other social media, will be addressed.

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8. *Clarence Brandenburg v. State of Ohio*, 395 U.S. 444, (1969).

### Freedom of Speech, Suicide, Media



### I41 Parental Alienation: Misinformation and Fake News

William Bernet, MD\*, Brentwood, TN 37027

**Learning Overview:** After attending this session, attendees will understand that widely distributed misinformation presents a threat to the integrity of forensic sciences. Parental Alienation (PA) will be used as an example, to show that misinformation regarding that topic has appeared in the popular press, presentations at conferences, journal articles, and books published by professional organizations.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving the evaluation of forensic clients (e.g., child custody evaluations) and improve the training of forensic psychiatrists (regarding PA).

Misinformation sometimes arises in the context of forensic psychology and psychiatry. In recent years, misinformation regarding the topic of PA has been expressed in peer-reviewed journal articles, presentations at scientific meetings, and books intended for mental health and legal professionals. PA is a mental condition in which a child—usually one whose parents are engaged in a high-conflict separation or divorce—allies himself strongly with one parent (the preferred parent or alienating parent) and rejects a relationship with the other parent (the target parent) without legitimate justification. The child's rejection of the target parent must be without justification for the child to be considered alienated; if a parent has been abusive or severely neglectful, the child's rejection of that parent is understandable and does not constitute PA. This presentation addresses and refutes three false statements that have been stated repeatedly.

The meme that "PAS is junk science" has taken on a life of its own. An influential psychiatrist, Paul J. Fink, stated in 2003 that Parental Alienation Syndrome (PAS) constitutes "junk science," and he repeated that opinion many times. In 2010, he reiterated—in his column in *Clinical Psychiatry News*—that PAS was "junk science invented by a psychiatrist."<sup>1</sup> After receiving letters objecting to Fink's statements, the management of *Clinical Psychiatry News* arranged for him to issue an apology and a clarification. Fink then said, "I do not deny that parental alienation occurs and that a lot of people are hurt when there is an alienator."

A common false allegation is that "Parental alienation has not been recognized by professional associations." In fact, mainline professional organizations have acknowledged the reality of PA through their publications, national and international meetings, and educational programs for their members. For example, the American Academy of Child and Adolescent Psychiatry (AACAP), the Association of Family and Conciliation Courts (AFCC), the American Psychological Association (APA), the American Academy of Pediatrics, and the American Professional Society on the Abuse of Children (APSAC) have published documents that explicitly recognize the reality and importance of PA.

Many critics of PA have alleged, "Parental alienation is not admissible in court under *Daubert* and *Frye* standards." Is PA admissible under *Frye v. United States*?<sup>2</sup> This rule merely requires that admissible expert opinion must be based on a scientific technique that is generally accepted as reliable in the relevant scientific community. Based on the information in this presentation, it can easily be shown that the concept of PA has been endorsed or accepted by numerous professional organizations. Is PA admissible under *Daubert v. Merrell Dow Pharmaceuticals*?<sup>3</sup> The factors to consider include: whether the theory or technique employed by the expert is generally accepted in the scientific community (explained previously in this presentation); whether the theory or technique has been subjected to peer review and publication (bibliographies and data bases include hundreds of articles from peer-reviewed journals); and whether the theory or technique can be and has been tested (has been accomplished in quantitative research).

While the clear majority of mental health and legal professionals who work with divorced families agree with the reality of PA, a small number of detractors of PA have generated a great deal of misinformation and fake news. Fake news appears to be endemic in Western and democratic societies, where a free press and online media encourage the transmission of both truth and falsehood.

#### Reference(s):

1. Fink, Paul J. Fink! Still at Large. *Clinical Psychiatry News*, 2010.
2. *Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923).
3. *Daubert v. Merrell Dow Pharmaceuticals*. US Supreme Court 509.U.S.579,113S.Ct.2786, 125L. Ed.2d 469. 1993.

#### Parental Alienation, Child Custody Evaluations, Child Psychiatry





### I42 Repressed Memory Syndrome Goes to Court: The Lopez Hearing

Vivian Shnaidman, MD\*, Skillman, NJ 08558

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**Learning Overview:** After attending this presentation, attendees will be familiar with: (1) repressed memory syndrome, (2) its legal implications, (3) how it might come up in a civil setting, and (4) the concept of the *Lopez* rule (referring to a hearing to determine whether or not the statute of limitations can be extended to a time when a victim actually learns about damages).<sup>1</sup> The interplay between repressed memory and damages has been a legal minefield in many landmark cases and now this study introduces the concept of a statute of limitations to further complicate the scenario and explore and understand how these factors all interact, utilizing a recent New Jersey case.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing previously unconsidered issues: (1) repressed memory, (2) damages to the victim, (3) the statute of limitations, and (4) understanding how appropriate forensic psychiatric evaluation and testimony can assist the legal system in making judicially appropriate decisions.

Repressed Memory Syndrome is defined as a situation in which an individual suddenly remembers an event, typically traumatic, that occurred years earlier. The literature has been divided on repressed memory, although conventional wisdom tells us that everyone has had memories that have been forgotten and then remembered. It is only when these memories arise and then become used in a legal arena that they become contentious.

A Statute of Limitations is a legal time limit beyond which legal action can no longer be taken in a court of law. For example, there is no Statute of Limitations on murder in the United States. Different crimes and different torts (civil wrongs) have different time limits. In New Jersey, child sexual abuse has a Statute of Limitations until age 23, two years after turning 21, the full age of majority, to file criminal charges.

In addition to criminal charges, individuals also have the right to file civil charges for damages, at least in the United States. In the case explored in this presentation, a 46-year-old man, JP, saw a photo on Facebook® on Thanksgiving Day 2014 of his cousin with “Uncle Jimmy” and after a moment of confusion, suddenly recalled, in vivid detail, two occasions on which his biological father sexually assaulted him.

This presentation will investigate the history of this case, the evidence JP presented to the civil court, his own psychiatric and personal history, and the testimony of the two opposing experts (with no identifying details). This author was brought in by the court as a tiebreaker, not to decide whether JP was actually molested, but on a matter of law: Was there sufficient evidence provided that the court should permit a *Lopez* hearing to extend the statute of limitations by an additional 21 years?

This presentation will review similar case law throughout the country and the world in case attendees find themselves in a similar situation one day and will refresh our repressed memories as to how to request and review a psychiatric-legal question from the attorney who hires you!

The story of this case is fascinating for many reasons and brings up the not trivial matter of how two extremely experienced and well-regarded experts could obfuscate to the point that the court needed to bring in an additional expert to explain all the jargon and help the court understand whether the Statute of Limitations should be extended.

To decide whether this case merited a *Lopez* hearing, the entire case was reviewed, and JP was evaluated. This method and the outcome will make an interesting group discussion for the attendees.

The surprise ending will be revealed to anyone who stays until the end!

**Reference(s):**

<sup>1</sup>. *Lopez v. Swyer*, 62 N.J. 267 (1973).

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**Repressed Memory, Statute of Limitations, Sexual Abuse**



## **I43 A New Classification of Exhibitionism**

*Anil Agrawal, MD\*, Maulana Azad Medical College, New Delhi 110002, INDIA*

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**THIS ABSTRACT WAS NOT PRESENTED.**



## Questioned Documents – 2019

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### J1 A Pre-Osborn Timeline of Events and References Concerning Handwriting Examination and Forgery

*Judith A. Gustafson, BS\*, Internal Revenue Service, Chicago, IL 60607*

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**Learning Overview:** After attending this presentation, attendees will be aware of pre-20<sup>th</sup> century events and references to handwriting identification, document examination, and forgery law.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of significant events concerning handwriting examination that occurred before the modern era of document examination.

In most standard document examination reference books, the history of document examination generally begins with Albert Osborn in the early 20<sup>th</sup> century. Osborn is widely considered to be the founder of modern document examination. Information prior to 1900 is treated briefly and spread among several sources.

This presentation will expand upon material presented in previous publications, providing a summary of these events and references in a brief, chronological format. This information may be of value in court testimony where document examiners may be asked about the history of their field.

The earliest mention of a law dealing with forgery may have occurred as early as 80 BC in ancient Rome with a law concerning the falsification of documents. In the 1500s and 1600s, the English Parliament created laws concerning forgery, with a “comparison of hands” said to be good evidence in such criminal cases.

Early courts sometimes relied on the use of “recognition witnesses”—people who were familiar with a given writer’s handwriting and would testify to it in court. The late 1700s saw the first specially qualified witness in an English-speaking court testify on a handwriting identification based on a direct comparison rather than on the use of a recognition witness.

While there is little written history of document examination in America until the late 19<sup>th</sup> century, there are references to an 1808 Louisiana law code that allows for an examination by “two persons having skill to judge of handwriting” and a recorded handwriting testimony in the early 1800s. In mid-century, a notable debate concerning handwriting examination occurred over the authorship of controversial Revolutionary War-era writings known as the Junius Letters. In Europe, handwriting examination also played a part in the “Dreyfus Affair,” a late 19<sup>th</sup>-century French political scandal.

Legal decisions of the 1880s and 1890s affirmed the importance of handwriting evidence and the use of experts and saw the first testimony on typewriter identification. Several early texts on handwriting examination were published in the decade of the 1890s by authors Daniel Ames, William Hagan, and Persifor Frazer, setting the stage for Osborn and the modern era of Document Examination.

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#### **Document Examination, Handwriting, Early History**



### J2 Education and Training in Forensic Document Examination: A Discussion of Issues and Ideas

Mara L. Merlino, PhD\*, Kentucky State University, Frankfort, KY 40601; Veronica B. Dahir, PhD\*, University of Nevada, Reno, Reno, NV 89557; Mauricio Alvarez, PhD, University of Nevada, Reno, Reno, NV 89557; Chris Sanchez, MA, University of Nevada, Reno, Reno, NV 89557; J. Guillermo Villalobos, MA, University of Nevada, Reno, Reno, NV 89557; Chris Swinger, MA, University of Nevada, Reno, Reno, NV 89557; Denise Schaar Buis, MA, University of Nevada, Reno, Reno, NV 89557

**Learning Overview:** After attending this presentation, attendees will understand how members of the field of forensic document examination view training and education practices. Current discussions of the advantages and challenges of changing training and education paradigms will be highlighted in the context of examiner opinions.

**Impact on the Forensic Science Community:** This presentation will impact the forensic sciences community by presenting conversations about the importance of creating systematic and standardized training programs with empirical and measurable benchmarks to demonstrate mastery and competence of trainees.

The National Institute of Standards and Technology (NIST) Organization of Scientific Area Committees (OSAC) has been engaged in efforts to improve the reliability and validity of the methods, procedures, and conclusions in all areas of forensic practice. OSAC discussions have included topics such as the cross-disciplinary standardization of reporting language; creation of clear, concise, and empirical standards for demonstrating expertise in the field; and meaningful, recognized certification or licensure. Among these discussions is the specification of programs of study that will qualify trainees to gain employment as experts in the field. Conversations around forensic document examination have included debates about the merits of modular training compartmented to a 24-month program of study; identifying a single recognized certifying organization; requiring certification for trainers/mentors; requiring private laboratories to adhere to the standards set for government labs; and standardization of document examiner education and training.

Previous research addressing the training and education of Forensic Document Examiners (FDEs) revealed that the most common factor that FDEs cited as a positive contributor to their training was access to high-quality materials ( $n=34$ ). For instance, the availability of textbooks, publications, and actual cases allowed FDEs to build upon the knowledge and experience of experts. Hands-on experience with trainers ( $n=22$ ) as well as working with highly skilled trainers ( $n=30$ ) were also among the most common things listed contributing to high-quality FDE training. Finally, FDEs reported that the repetition inherent in working through many cases gave FDEs experience that directly applied to work that they would be conducting in the field ( $n=18$ ). A few participants ( $n=4$ ) indicated that being taught to take a conservative approach to document examination ensured that their opinions and conclusions were defensible and supported by evidence.<sup>1</sup>

This presentation will compare the results of a recent survey of the education and training background of professional document examiners (National Institute of Justice Award # 2015-DN-BX-K069) with results from the 2014 survey, highlighting similarities and differences in their views on current training and suggestions for improvement. The current movement toward an educational model of training standards and practices will be discussed.

Further discussion will center around the ongoing discussions in the field about creating standardized training programs that incorporate the identification of relevant knowledge, skills, and abilities; creating measurable and objective course goals; specifying learning objectives that incorporate introductory-, intermediate-, and mastery-level goals; creating valid and reliable measures of learning; and creating objective and measurable benchmarks for determining training effectiveness. This discussion will include information about identifying constructs to be measured, how to measure the reliability and validity of assessment techniques, and constructing standardized tests and measures.

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#### Professional Training, Education Models, Professional Development



### J3 Evaluation of Gray Value Measurements of Visual Spectral Data to the Characterization of Alcohol-Based Inks From Colored Felt-Tipped Blending Markers

*Clarra G. Moore, BS\*, Sam Houston State University, Huntsville, TX 77340; Patrick Buzzini, PhD, Sam Houston State University, Huntsville, TX 77340*

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**Learning Overview:** After attending this presentation, attendees will understand the use of gray value measurements to objectively detect and compare the most discriminating optical properties of colored felt-tipped blending markers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a simple method for analyzing a previously unstudied type of ink, expanding upon the limited knowledge of felt-tipped pens. The proposed protocols in this research can be used by questioned documents examiners to characterize these types of samples.

Ink analysis began in the early 1900s with fountain pen ink comparisons and has since expanded into one of the most researched areas in the questioned document field. Pen ink analysis includes determining the origin and components of the ink, comparing known and unknown samples, and dating ink samples. Ink analysis typically serves to authenticate documents and/or analyze any alterations. Most pen ink research concentrates on ballpoint pens and, more recently, gel pens, both of which are the most common types of pens encountered in questioned document examinations. Fiber- and felt-tipped pens first appeared in the mid-1900s and now are commonly used in artistic fields, such as drawing, graphic design, crafting, and calligraphy.

The felt-tipped markers used in this study were Copic® Sketch markers from the Japanese company Too Corporation. Initially, Copic® created markers for the manga industry. However, they are now being used in a wide variety of applications. Copic® markers are valued for their long shelf life, replaceable felt-tipped nibs, and refillable ink cartridges. The color name system has three main parts. The first part of the name is a letter or letters denoting color family, the second part is a numeric value of saturation, and the third part is a numeric value of brightness. Copic® Sketch markers are one of four types of markers sold by Copic®. The Copic® Sketch markers come in 358 different colors, as well as three types of nibs. They are also marketed to have the blending characteristics of watercolor paint. The applications of these markers vary from manga and comics to fashion and interior design.

Samples were prepared by marking one ink stroke on regular white office paper. Forty-two alcohol-based inks from colored felt-tipped blending markers were analyzed based on visual spectral data obtained from a Visual Spectral Comparator (VSC), and gray value measurements were recorded for purposes of differentiation. The types of markers studied have never been comprehensively researched, and no published research was found in the literature on the possibility of using the gray values gathered from images to analyze inks. It is hypothesized that the combination of illumination types and recorded gray values can be used to objectively distinguish between different inks in the context of comparative examinations. The present portion of this study deals with the implementation of the method of objectively comparing gray value data from alcohol-based blending marker inks.

The 42 ink samples were analyzed using various illumination types (i.e., Infrared (IR) reflectance, IR luminescence, and Ultraviolet (UV) fluorescence) and filters ranging from the visible to the near-IR range (up to 850nm) to detect the optical properties of the samples. The optical properties of the ink samples were observed. Standardized color checkers and images of samples collected in IR absorbance and/or luminescence modes were then analyzed using ImageJ to extract ten replicate gray values of the samples. The images of the ink samples could be separated into four main groups, depending on the samples' optical characteristics. Within these four groups, most comparisons of ink stroke pairs could be visually distinguished from each other based on their optical properties. Thirteen inks showed completely distinctive optical characteristics. Based on the standardized color checkers, gray values ranged from about 24 to 235. Many ink pair comparisons were discernible based on the gray values of at least one illumination source and filter. A few ink samples had overlapping ranges of gray values, which led to difficulty distinguishing between samples. The method of using the VSC6000 combined with ImageJ gray value analysis shows potential in distinguishing between ink samples from colored felt-tipped blending markers.

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#### Questioned Documents, Ink Analysis, Blending Markers



### J4 Laser Printer Identification: A Real Case Study

*Jasna Galekovic\**, Forensic Science Centre Ivan Vucetic, Moi, Zagreb 10000, CROATIA

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**Learning Overview:** The goal of this presentation is to inform attendees of the possibilities of identification of a laser printer used for producing counterfeit banknotes, based on specific printer defects.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that individual defects of laser printers can be used as tools for the identification of specific laser printers used to create counterfeit banknotes or documents.

Due to their widespread use and availability, laser printers are often used as tools to produce counterfeit documents and banknotes. Forensic document examiners are often faced with requests to determine if seized counterfeit documents or banknotes were produced with a seized laser printer. Identifying the possible source of printed documents or banknotes can be quite a difficult and demanding task for forensic examiners.

An actual case study is presented in which police investigators found a counterfeit banknote and, during the investigation, a laser printer was seized as the possible instrument for producing the counterfeit banknote. The objective of this examination was to determine if the counterfeit banknote was produced by the seized laser printer.

The examination was performed using non-destructive methods—video-spectral and stereomicroscopic analysis. After detailed examination of the counterfeit banknote, it was concluded that the counterfeit banknote was produced by a device (laser printer or photocopier) using Cyan, Magenta, Yellow, and Black (CMYK) colored dry toner. On the front side of the counterfeit banknote, in the lower left corner of the paper, the specific traces of CMYK toner particles were found. Those toner particles were in the form of several parallel lines and looked like the traces of a specific printer or photocopier defect marks.

The next step of the examination process was to take test samples from the seized laser printer. After detailed examination of those samples, identical marks were found, repeated in the same position and with the same distance on all samples. The fact that the distance between those marks was constant on all samples led to the presumption that they were the result of photosensitive drum defects. The photosensitive drum was taken out of the printer and, after detailed microscopic examination, the specific scratches on the drum surface were found. The defects on the drum surface were compared with the specific clusters of toner particles that were present on the counterfeit banknote, and their shape was identical. Based on those findings, it was possible to determine that the seized laser printer was used for producing the counterfeit banknote.

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#### Laser Printer Identification, Drum Defects, Counterfeits



### J5 Measuring the Frequency Occurrence of Handwritten Numerals: An Expanded Database

Thomas W. Vastrick, BS\*, Apopka, FL 32703; Ellen M. Schuetzner, BA, Chicago, IL 60646-3728; Kelsey Osborn, Forensic Science Institute, Edmond, OK 73034

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**Learning Overview:** After attending this presentation, attendees will be informed of the most recent frequency occurrence measurements for the features added to the overall handwriting database. Attendees will know about the expanded database and understand the quality control processes that are the bases for the integrity of these studies.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing expanded foundational and statistical bases for associations of known handwriting specimens to writings in question.

Beginning in 2009, members of the forensic document examination community began making a concerted effort to design and implement a course of study to develop statistically based frequency of occurrence proportions of handwriting, hand printing, and numerals for use in handwriting comparison analyses. The group formed after noting several court rulings in which judges admitted handwriting comparison in court but noted concern over the limited foundational bases for the axioms of the science. In 2010, two document examiners and two statisticians received a grant from the National Institute of Justice to undertake the first of the frequency occurrence studies. This study was formally presented at the 2015 American Academy of Forensic Sciences (AAFS) Annual Scientific Meeting in Orlando, FL, and published in 2017.<sup>1</sup>

There had been several studies in the past, most of which were of limited scope. It was also noted that there was little in the way of population sampling design built into the previous studies. The statisticians worked diligently to design an appropriate protocol for population sampling based on published material concerning intrinsic and extrinsic factors in handwriting and how those factors could, and should, be addressed in the collection process. A protocol was developed and overseen by the team during the first study.

In addition, the statisticians developed a protocol for quality assurance by utilizing attribute agreement analyses into the study protocols. These analyses studied the potential of variance by multiple forensic document examiners, or by one forensic document examiner, over time. It was decided that any feature used in the database must pass the protocol with a 100% score; in other words, no deviation of results scored by the assessors/forensic document examiners.

To date, the results have been approximately 1,000 features having been studied for frequency occurrence proportions within the United States. Several past studies have concluded that the pattern recognition areas of the forensic sciences should report conclusions based more on a statistical foundation than on experience alone. The development of a frequency occurrence proportion database is designed to directly address that recommendation. The results of the first two studies have been reported in court with resounding success. Additional studies have been mapped out for the purposes of expansion of the database. This expansion includes additional features and a larger population sampling.

This study is the third frequency occurrence study. Both the first and second studies have been published in the *Journal of Forensic Sciences*, the second being published in 2018. It is the purpose of this study to begin to expand the existing handwriting database to provide a larger foundation of statistical study into the heterogeneity of handwriting features.

#### Reference(s):

- <sup>1</sup> Ellen M. Schuetzner, Thomas W. Vastrick, Mark Johnson, Kevin P. Kulbacki. Frequency Occurrence of Handwriting and Hand Printing Characteristics. J3. *Proceedings of the American Academy of Forensic Sciences*, 67<sup>th</sup> Annual Scientific Meeting, Orlando, FL. 2015.
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#### Frequency Occurrence, Numerals, Statistics



### J6 Cognitive Human Factors and Forensic Document Examiner Methods and Procedures: Key Results From an International Study of Handwriting Experts

Mara L. Merlino, PhD\*, Kentucky State University, Frankfort, KY 40601; Veronica B. Dahir, PhD\*, University of Nevada, Reno, Reno, NV 89557; Derek L. Hammond, BA\*, U.S. Army Criminal Investigation Laboratory, Forest Park, GA 30297-5205; La'Quida Smith, MA\*, Kentucky State University, Frankfort, KY 40601; Chandler D. Al Namer, BA, Kentucky State University, Frankfort, KY 40601; Taleb A. Al Namer, BA, Kentucky State University, Frankfort, KY 40601; Mauricio Alvarez, PhD, University of Nevada, Reno, Reno, NV 89557; J. Guillermo Villalobos, MA, University of Nevada, Reno, Reno, NV 89557; Charles Edwards, MA, University of Nevada, Reno, Reno, NV 89557

**Learning Overview:** After attending this presentation, attendees will understand some of the principles of cognitive human factors, cognitive psychology, and the use of eye-tracking technology to study attention and feature-matching processes as they relate to decision-making processes in forensic document examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by addressing the relationship between cognitive human factors such as context effects, information about the measurement characteristics of the currently used nine-position authorship opinion scale, and findings about sufficiency in the number and complexity of handwriting samples available in signature comparison tasks.

The International Ergonomics Association defines human factors as “the scientific discipline concerned with the understanding of interactions among humans and other elements of a system, and the profession that applies theory, principles, data, and methods to design in order to optimize human well-being and overall system performance.”<sup>1</sup> Cognitive ergonomics is a subfield of human factors in which cognitive processes, such as memory, perception, reasoning, decision making, skilled performance, and human reliability, are examined in the context of work and operational settings. The goal of cognitive ergonomics is to improve task performance by the systematic study of the interaction between human cognitive functioning and the systems or environments in which tasks are performed.

Forensic science is produced and consumed in the context of various systems developed by human actors. At the “Examiner Action” level, error may be based on examiner decision (e.g., failure to recognize exemplars or standards as inadequate, or insufficient data to support conclusions); examiner perception (e.g., not all data are perceived, or data are inadequately compared); examiner skill (e.g., data are misinterpreted or misunderstood); or examiner violations (e.g., disregarding procedures, or misrepresenting information).<sup>2</sup>

“Conditions that Affect Performance” are adverse mental states of the examiner (e.g., anger, haste, mental fatigue, stress, or other cognitive factors); adverse physiological states (e.g., illness, lack of sleep, eyestrain); physical or mental limitations of the examiner (e.g., inadequate training, limited experience, color vision deficiency); communications (e.g., confusing or conflicting directions or demands, inadequate communication among examiners, inadequate report-writing skills); physical environment (e.g., clutter, inadequate lighting, poor workplace design); or the technical environment (e.g., defective, inadequate, or outdated software, tools, or equipment).

Interdisciplinary research encompassing expertise from forensic practice, social and cognitive psychology, vision science, and other areas is needed to establish the basis and extent of expertise, to develop rigorous protocols and measures, and to establish education and training programs that consistently and comprehensively address the knowledge and skills required to establish expertise in forensic fields. A human factor approach to understanding issues of reliability, validity, proficiency, expertise, and sources of bias involves an examination of multiple sources of information, and it is important to address the production of forensic science from multiple perspectives. This international multidisciplinary research program (National Institute of Justice Award No. 2015-90606-KY-DN) extended previous research exploring the reliability, measurement validity, and accuracy of established Forensic Document Examiner (FDE) procedures. The three experiments in this research investigated the following questions: (1) What is the relationship between the context established by presentation order of questioned and known writing and the examination process?; (2) How do examiners apply the currently used bipolar continuum of certainty (Elimination through Identification with a center position of Inconclusive) when expressing their opinions about the authorship of questioned writings?; and (3) How much writing constitutes “sufficient” information upon which to base an opinion?

This series of four papers will report on the results of: (1) a telephone survey about the training, education, and background of project participants, as well as their opinions about the strengths and weaknesses of professional training; (2) an eye-tracking experiment investigating the relationship between position of the questioned and known writings and the visual inspection of available writing features; (3) examiners’ use of the nine-position “authorship” scale; and (4) an eye-tracking experiment investigating examiner call accuracy given varying numbers of writings and writing complexity.

#### Reference(s):

1. <http://www.iea.cc/whats/>.
2. Working Group for Human Factors in Handwriting Examination. *Forensic Handwriting Examination and Human Factors: Improving the Practice Through a Systems Approach*. U.S. Department of Commerce, National Institute of Standards and Technology. 2018.

#### Cognitive Human Factors, Empirical Measurement, Sufficiency and Complexity





### J7 A Methodology for the Removal of Adhesives

*Kevin P. Kulbacki, MSFS\*, Internal Revenue Service, National Forensic Laboratory, Chicago, IL 60607*

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**Learning Overview:** After attending this presentation, attendees will understand a basic methodology for the removal of adhesives, such as packaging tape.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a case study for when the removal of an adhesive substance may be required. Additionally, this presentation will include a basic methodology for conducting the removal.

Forensic document examiners occasionally conduct examinations on documents that have adhesive products, such as tape. This presentation arises from casework in which the evidence consisted of makeshift address labels. The makeshift address labels were consistent with cut-out sections that were subsequently taped onto the envelope. During the investigation, sheets of paper with an assortment of addresses were recovered with sections cut out, consistent with address labels. Research was undertaken to determine the reliability of using un-du® Sticker, Tape and Label Remover to remove tape from envelopes without damaging the underlying paper labels to allow for subsequent fracture match examinations.

Test samples were designed recreating the circumstances surrounding the evidence in question. Initial testing was conducted under a fume hood using the shovel-like tool that is built into the un-du® bottle. Following the included directions, a small amount of un-du® was applied to the built-in tool near the corner of the tape. The tool was then used to scrape underneath the tape. While following this procedure, it was determined that the bottle would continue to drip more solution than what was needed, thereby leading to saturation of the document. Additionally, it was determined that if an excess of un-du® solution was applied, it would possibly stain the document.

The methodology was then refined to remove the use of the built-in tool to prevent saturation of the document. Under the modified methodology, a spoonula was used to apply the un-du® solution at a slower pace and in a more refined manner. Using this method, it was possible to apply smaller amounts of the un-du® solution directly underneath the tape as it was being removed. Through careful application of this methodology, it was possible not only to separate the tape from the envelope, but also to separate the makeshift label from the tape at the same time.

It was determined that under controlled circumstances, un-du® was effective at allowing the removal of tape with little-to-no staining of the documents in question.

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#### Methodology, Removal, Adhesives



### J8 Analysis of Black Toners Using Scanning Electron Microscopy (SEM)

*Anna Lasinska\*, Forensic Bureau Internal Security Agency, Warszawa, AE, POLAND*

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**Learning Overview:** After attending this presentation, attendees will understand some principles of printed matter examination and identification. The goal of this study was the estimation capabilities of the verification of toners on printouts made by using laser printing devices and laser multifunctional devices.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing a novel method for the identification of laser printing. The proposed method could prove crucial for forensic casework and could run parallel to predominant document analysis methods. Hence, the present technique can be utilized for building a large database of various toner references for future considerations. The classification of the toner samples can be further studied using other analytical methods, such as Fourier Transform Infrared (FTIR) and Raman spectroscopy. These methods may help the forensic science community to characterize and classify the toners and present the data as additional information to the judicial system for the verdict.

The universal availability of printers and multifunctional devices has resulted in a decrease in handwritten documents. Since we currently have a choice of different types of printers, multifunctional devices, and copiers, the research to identify a printing device is a relevant problem.

To date, a few reports have indicated the possibilities of varying the available toners in Poland, based on studies by Infrared (IR) spectroscopy and Scanning Electron Microscopy (SEM). However, based on this study, it was not clear how to relate the toner to the appropriate device (printer, copier).

Therefore, the identification of printing and copying devices was performed within the framework of a research project by the Forensic Bureau of the Internal Security Agency. The goal of the study was to determine if a laser printer or multifunctional laser device could be identified by an examination of the toner on a printout from that device. The research materials used were printouts from ten different companies.

An analysis of the toners on printouts made from 134 models of laser printing devices was conducted. This analysis showed that the implementation of a more sensitive method such as SEM created new opportunities for the identification of laser printing. Group selection has been made and, in some cases, individual printouts. The present technique can be utilized for building a large database of various toner references for future consideration. However, the classification of the toner samples can be further studied by using other analytical methods such as FTIR and Raman spectroscopy. These methods may help the forensic science community to characterize and classify the toners and present the data as additional information to the judicial system for the verdict.

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**Toner, SEM, Analysis**



### **J9 An Interdisciplinary Study: Alcohol and Its Influence on Breath Alcohol Concentration (BrAC), Blood Alcohol Concentration (BAC), and Handwriting**

*Andrea Ledic, BS\*, Croatian Forensic Science Centre "I. Vucetic," Zagreb, Europa 10000, CROATIA*

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**Learning Overview:** After attending this presentation, attendees will better understand the influence that alcohol may have on a writer's handwriting signature.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a unique interdisciplinary case study in which handwriting was analyzed and the results compared to BrAC and BAC, as well as by teaching attendees about the effects alcohol has on the brain and nerves that may result in the writer's handwriting and signature containing characteristics of distortion.

Alcohol is a substance that is toxic to the human body, primarily the brain, and it directly influences human behavior. Ethanol can be found in alcoholic beverages. It is a modern poison that has an effect on the body's Central Nervous System (CNS) similar to that of anesthetics, such as ether or chloroform.

Previously published papers were primarily based on the analysis of the characteristics of handwriting and signatures of persons under the influence of alcohol with no significant statistical analyses. The goal of this research was to establish the significance of measuring the influence of alcohol in relation to blood, breath, and handwriting to carry out a statistical analysis of the collected data.

To verify the collected data, a controlled drinking study was conducted with 63 healthy volunteers (21 male and 42 female participants between the ages of 26 and 61 years) with a body mass index lower than 30. The participants consumed alcoholic beverages to simulate real drinking conditions.

Handwriting samples were taken before, immediately after, and an hour following alcohol consumption. They were examined under the VSC 6000 HS and Leica® stereo microscope.

The Dräger Breath Alcohol Analyser, model 6810Za, was used for breath analysis before and 15 minutes after alcohol consumption. Blood samples taken before and an hour after alcohol consumption were analyzed using the Perkin® Elmer® Gas Chromatograph with an ionization detector (Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID-HS)) and TurboMatrix Headspace Auto Sampler.

The BrAC is the amount of alcohol in breath, while the BAC is the amount of alcohol in blood. No significant differences were found between the BrAC and BAC results obtained from a single participant. However, the same amount of the same alcoholic beverage shows different BACs in taller and heavier individuals as opposed to shorter and lighter individuals because the organism of taller and heavier persons contains more water, which dilutes the alcohol they consume.

Taking practice and theory into consideration, the changes visible in the handwriting of persons who are under the influence of alcohol are proportionate to BAC, but the effects of alcohol on handwriting may vary depending on the individual's health and their physical traits. Regarding the handwriting, the results obtained indicate that, in certain cases, handwriting characteristics, such as the size of the letters/words, length of syllables/words, space between letters/words, tremor, as well as others, significantly increase under the influence of alcohol. Handwriting was also compared to the results obtained by the Dräger test and blood sample analysis with the aim of establishing whether handwriting can be used for obtaining an accurate measurement of BAC of the person writing.

The results of this research may also be used in certain cases for establishing the effects of alcohol on a person's ability to drive, considering that the same part of the brain is responsible for writing and driving.

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**Handwriting, Breath Alcohol Concentration, Blood Alcohol Concentration**



### J10 The Classification of Raman Patterns From Inkjet Printer Inks Using Visual Comparisons of Spectra and Different Statistical Methodologies

Patrick Buzzini, PhD\*, Sam Houston State University, Huntsville, TX 77340; James M. Curran, PhD, University of Auckland, Auckland 1142, NEW ZEALAND; Carrie Polston, BA, Sam Houston State University, Huntsville, TX 77341

**Learning Overview:** After attending this presentation, attendees will understand the criteria to differentiate Raman patterns measured on inkjet-printed documents from different sources and the ability of different multivariate statistical methods to classify the collected Raman spectra.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing the comparison between traditional visual inspection and different statistical methods to differentiate and classify spectroscopic patterns in an investigative context.

Inkjet printers are devices that are commonly encountered in society. Therefore, it is not surprising that they are likely to be involved in different types of illicit activities, such as threats or extortions by means of anonymous letters, fraud in the context of disputed contracts, alterations to or counterfeit of ID documents, or counterfeit of currency. The printing process used in inkjet technology involves the production of a constellation of ink dots of micrometric size that are projected without contact to the receiving surface (i.e., paper). When coupled with a microscopical approach, Raman spectroscopy has been demonstrated to be a suitable method for obtaining a chemical signature *in situ* from the three main colored components (cyan, magenta, and yellow) of inkjet printer inks. Although the Raman technique is already a relatively well-established method for the characterization of colorants (both dyes and pigments), this study highlights the contribution of minor peaks within Raman spectra to improve the discriminating capabilities of the technique.

In the present phase of this project, 231 Raman spectra were collected from the cyan, magenta, and yellow color components of 11 inkjet printer ink samples provided by the Counterfeit Forensic Section of the United States Secret Service, using a Near-Infrared (NIR) laser wavelength at 785nm. Spectra were first compared visually, and groupings were formed for each individual color and for the three colors jointly considered. Since the overall goal of this project is to evaluate if Raman data gathered from these three components constitute, together, a chemical signature of sufficient discriminating quality to provide reliable investigative leads, many spectral comparisons need to be carried out. However, visual comparisons are impractical and tedious for this purpose, and a sensible statistical classifier is then required for conducting spectral comparisons. To facilitate the implementation of a statistical approach, two main problems need to be considered. The first is that most differentiations between spectra of different inkjet printer ink samples are made by consideration of minor peaks since most of the Raman bands present in a spectrum are shared by multiple samples. This is due to the prevalent use of few colorants in the inkjet manufacturing industry and/or to the higher scattering property of these colorants compared to other present within the ink formulation. The second problem is that Raman spectra often exhibit large intra-source variation with regard to the absolute intensity of the signal, although their relative intensities and wavenumber positions present high repeatability. This implies that appropriate data pre-treatment and feature selection must be carefully investigated.

In this study, spectra have been submitted to baseline correction, and four normalization methods have been investigated: normalization to the same frequency, normalization to area unity, normalization to unit sum, and Standard Normal Variate (SNV). Data dimension reduction techniques of Principal Component Analysis (PCA) and t-Stochastic Node Embedding (t-SNE) have been utilized for visualizing the collected high-dimensional spectral data. The potential of the classification methods of Linear Discriminant Analysis (LDA), random forests, and Naïve Bayes classifiers were evaluated. At this stage, none of the selected combinations of statistical methods resulted in the classifications observed by visual inspection of spectra. For example, for random forests (applied after normalization to area unity and t-SNE), ten-fold repeated cross-validation with three repeats per fold were used to achieve an overall accuracy of the model of approximately 75% for the cyan color. A following step of this study will consist of selecting lower portions of the spectral range that are expected to yield less inter-sample redundancy.

This research is funded by an award from the National Institute of Justice.

#### Questioned Documents, Raman, Inkjet



### J11 Writing Speed and Fluidity and Accuracy of Calls in High and Low Complexity Signature Comparisons

Mara L. Merlino, PhD\*, Kentucky State University, Frankfort, KY 40601; Mauricio Alvarez, PhD, University of Nevada, Reno, Reno, NV 89557; Taleb A. Al Namer, BA, Kentucky State University, Frankfort, KY 40601; J. Guillermo Villalobos, MA, University of Nevada, Reno, Reno, NV 89557; Charles Edwards, MA, University of Nevada, Reno, Reno, NV 89557

**Learning Overview:** After attending this presentation, attendees will understand the relationship between writing speed and fluidity, writing complexity, and the accuracy of decisions made by forensic document examiners about whether the signatures are genuine or simulated.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the importance of engaging in theoretically based, multidisciplinary research to an understanding of the nature of the methodology and expertise in forensic document examination.

The extensive scrutiny of the methods and findings of numerous areas of expert testimony has prompted acrimonious debate among academicians, forensic practitioners, and legal professionals concerning what has been referred to by the Forensic Science Committee of the National Academy of Sciences (“Committee”) as “faulty forensic science analyses.”<sup>1</sup> While acknowledging the importance and utility of the forensic disciplines, the Committee also addressed the perceived flaws in such evidence. For example, advances in technology in various forensic disciplines, especially in the field of DNA testing, show that erroneous or misleading forensic evidence has contributed to the wrongful conviction of innocent individuals.<sup>1</sup> The Committee called for improvements in forensic science practices, arguing that increased and demonstrated reliability and validity in forensics will help law enforcement investigations by improving the reliability of identifications, and homeland security efforts will also improve as improvements are made in the methods and procedures of the forensic disciplines.<sup>1</sup>

The Committee specifically identified several important issues, including practitioner certification, accreditation, and the availability of skilled, well-trained personnel.<sup>1</sup> Many areas of forensic science lack uniformity in training, accreditation, and practice standards. The Report stated that operational principles and procedures for many disciplines are not standardized between or within jurisdictions; attempts at standardization are not viewed favorably in many instances; and that protocols such as Scientific Working Group (SWG) standards “often are vague and not enforced in any meaningful way ... These shortcomings obviously pose a continuing and serious threat to the quality and credibility of forensic science practice.”<sup>2</sup>

The Committee also discussed the lack of demonstrated validity and reliability within the interpretation-based disciplines, stating “... no forensic method has been rigorously shown to have the capacity to consistently, and with a high degree of certainty, demonstrate a connection between evidence and a specific individual or source ... The simple reality is that the interpretation of forensic evidence is not always based on scientific studies to determine its validity. This is a serious problem. Although research has been done in some disciplines, *there is a notable dearth of peer-reviewed, published studies establishing the scientific bases and validity of many forensic methods.*”<sup>3</sup>

Among the writing characteristics identified by professional document examiners as indicators of genuine writing are the speed and fluidity of the questioned writing when compared to the known writings of an individual. This presentation reports empirical information about the accuracy of results of forensic document examiner comparisons of genuine-to-genuine and genuine-to-simulated signatures when considering the handwriting dynamic information obtained from digitizing tablets using Movalyzer<sup>®</sup> software. Results comparing high-complexity and low-complexity signature samples will also be reported.

#### Reference(s):

1. National Research Council. 2009. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/12589>.
2. id.
3. *Supra* note 1.

#### Decision Accuracy, Writing Speed, Signature Complexity



### J12 Trends in Forensic Document Examination in Québec, Canada

*Mylene A. Signori, BSc\*, LSJML, Montreal, PQ H2K 3S7, CANADA; Richard K. Van Gheluwe, BSc, Montreal, PQ H2G 2X8, CANADA*

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**Learning Overview:** After attending this presentation, attendees will understand the types of criminal cases which are submitted to the Québec Forensic Provincial (state) Laboratory, over the past ten years. Some questions had arisen concerning the shift in the types of analysis that may have taken place in recent year in forensic document examinations in relation to police investigative practices and judicial practices. Ultimately, some changes were observed.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting an overview of the capacities of a francophone forensic document examination unit in North America, over a period of ten years.

Over the past ten years, there seems to have been a rising pressure on the Canadian justice system in terms of delays. These delays may be attributed to different factors: the lack of rooms for the trials, the shortage of staff in the judicial system, the prosecutors, the defense lawyers, and the overload of the judges' agendas. These delays have also been ascribed to the forensic analyses.

The Laboratoire de Sciences Judiciaires et de Médecine Légale wanted to find out if this intangible pressure would result in a shift in police investigators' requests for analysis in the forensic document examination unit. In all, several individual investigative requests (in forensic document examination only) were examined, pertaining solely to criminal cases over a period of ten years (2006 to 2017). The Statistical Package for Social Sciences data-collecting software was used to compile the studied elements. Civil and private cases were not retained for this study.

The cases were assessed under different criteria, including the time of year the request was received and completed, the type of offenses, the number of documents to analyze in each case, the time taken to entirely process the case, the complexity of the examinations, etc.

Originally, approximately 30 different criminal offenses were listed. These had to be grouped into main categories for this research: financial crimes, misdemeanor and threats, crimes against the persons—major crimes, crimes against property, organized crime, terrorism, and other infractions.

In July 2016, the Canadian Supreme Court rendered a judgement, *R. v. Jordan*, which stated new delays under which criminal and civil cases had to be dealt with diligently—from the beginning to the end of the judicial process—before being detrimental to the accused.<sup>1</sup> Although this judgement is fairly recent, a shift in requests has already been observed, resulting in more pressure being imposed upon all forensic analysts.

This research ultimately demonstrated that a slight change in forensic document examination requests has been observed during recent years, in the type of cases submitted, the complexity of the cases submitted by the combined investigations joint task forces, and the gravity of the offenses.

**Reference(s):**

<sup>1</sup>. *R. v. Jordan*, 2016 SCC 27 [2016] 1 S.C.R. 631

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**Document Examination, Trend, French-Speaking**



### J13 The Examination of Crossed Lines for the Determination of Sequence of Strokes Using Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) and X-Ray Fluorescence (XRF)

Samiah Ibrahim, BSc\*, Ottawa, ON K1S 1R1, CANADA; Julie Binette, BS\*, Canada Border Services Agency, Ottawa, ON K2E 7M6, CANADA; Mario Larouche, BS, Ottawa, ON K2E 7M6, CANADA

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**Learning Overview:** The goal of this presentation is to provide information about a novel approach to the determination of sequence of stroke for crossed ink lines.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding to the present knowledge base concerning the determination of sequence of strokes for crossed ink lines.

During the course of forensic examinations of documents, the question of sequence of strokes is common. Determining which ink or printed line was produced first, second, third, etc. may play an important role in answering questions about the genuineness or veracity of the document or its content. Furthermore, this problem is growing in scope as the number of writing instrument types, ink formulations, and printing devices and media expands. Forensic Document Examiners (FDEs) may be presented with line crossings of any kind, and so need in their arsenal the best fit analysis for the possible mixed-media before them.

Over the years, many destructive and non-destructive techniques have been developed for or applied to the examination of crossed lines; Rodrigues e Brito et al. have published a comprehensive review of the research to date in this regard.<sup>1</sup> The methods outlined in this compendium include microscopy, lifting techniques, the use of electrostatic detection devices, chemical analyses, chemometric, and advanced imaging techniques. While this presentation outlines the use of XRF techniques to resolve problems of crossed lines, there is little research on the application of LA-ICP/MS.

This research explores the use of LA-ICP/MS and XRF as semi-destructive and non-destructive techniques, respectively. LA-ICP/MS is a highly sensitive analytical chemistry technique used to determine the trace element concentration and elemental distribution of a wide range of solid samples. This technique can be considered semi- or micro-destructive in that, with an appropriate stage, a document need not be cut or otherwise destructively manipulated. However, the technique uses a laser to directly ablate, or burn, the sample surface for a total volume of <1µg, thereby ensuring the integrity of the sample and allowing for other measurements using different techniques. XRF is a non-destructive analytical technique in which a primary X-ray beam is used to excite fluorescent radiation from the sample, yielding an elemental map of the region of interest.

Twenty-five writing instruments of various ink formulations were used to create intersecting lines with printed matter from five devices and with paper designed for use with the printing devices (e.g., inkjet, photocopy, laser papers). The crossed lines were created such that the sequence was cataloged and blinded from the rest of the study team. The samples were imaged, observed microscopically and macroscopically, then run using both techniques. The resultant analytical output and crossed lines were examined by 5 FDEs and 20 non-examiners to determine if: (1) the individuals could easily and reliably determine the sequence of strokes based on the output, and (2) there was a difference between the results from the FDEs and laypeople. Notwithstanding the complexity of intersecting line problems, this study adds to the body of research to determine reliable techniques for use by FDEs in casework.

#### Reference(s):

- <sup>1</sup> Livia Rodrigues e Brito, Angelica Rocha Martins, Andre Braz, Amanda Belem Chaves, Jez William Braga, Maria Fernanda Pimentel. Critical Review and Trends in Forensic Investigations of Crossing Ink Lines. *Trends in Analytical Chemistry* 94 (2017): 54-69.

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**Questioned Documents, Forensic Chemistry, Crossed Line Intersections**



### J14 The Comprehensive Analysis of Counterfeit Currency

*Irina Geiman, MS\*, United States Secret Service, Washington, DC 20223*

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**Learning Overview:** After attending this presentation, attendees will understand the need for a comprehensive examination of highly deceptive counterfeit currency and the timely dissemination of the findings to key stakeholders.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by advancing the understanding of chemical and instrumental analysis techniques as they pertain to the examination of counterfeit currency. This presentation will discuss analysis and comparison of materials found in counterfeit Federal Reserve Notes originating on three different continents.

Counterfeit United States currency is found throughout the world but is often of poor quality and is easily detected by cash handlers and banknote scanning equipment. However, highly deceptive counterfeit Federal Reserve Notes occasionally appear in circulation and provide an excellent opportunity to study the techniques used by criminal elements to reproduce currency components, such as paper, inks, and security features. Understanding the materials and methods employed by the counterfeiters allows the United States Secret Service (USSS) to investigate the relevant supplies, manufacturing, and distribution networks.

Depending on the sophistication level of the counterfeiting efforts, the notes may contain printing inks of the types found in genuine Federal Reserve Notes. The intaglio inks, metallic inks, and optically variable inks are of particular interest to USSS as they may provide information about the intended distribution routes of the counterfeit notes. For example, the presence of optically variable ink with a well-simulated color change indicates intent for person-to-person distribution, while the presence of magnetics indicates intent to deceive banknote scanners. Counterfeit notes may also contain custom paper incorporating counterfeit security fibers, security threads, and watermarks.

Access to a full suite of analytical instrumentation allows the USSS Counterfeit Forensic Section Document Analysts to conduct in-depth profiling of the materials found in counterfeit United States currency. For example, Fourier Transform Infrared (FTIR) spectroscopy may be utilized to analyze polymers of counterfeit security fibers, security threads, and security ribbons. X-Ray Fluorescence (XRF) spectrometry may provide valuable information about the elemental composition of the paper and ink while Microspectrophotometry (MSP) is essential for examination of the color gamut of counterfeit materials.

Examination of the individual counterfeit currency components provides information on the types and the consistency of the employed materials plus allows the USSS to build an overall picture of the counterfeiter's skills and potential motivations. Such information is then disseminated to the key stakeholders to be able to forensically link counterfeit notes and cases and to assist investigators in developing timely leads. It also helps the United States Currency Program members study the reliability and effectiveness of the current genuine security features.

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#### Currency Analysis, Counterfeit Currency, Federal Reserve Note





### J15 Assessing the Impacts of Induction Spatial Effects on Magnetic Flux Measurements of Toners

*Carrie Polston, BA\*, Sam Houston State University, Huntsville, TX 77341; David E. Thompson, PhD, Sam Houston State University, Department of Chemistry, Huntsville, TX 77341; Patrick Buzzini, PhD, Sam Houston State University, Huntsville, TX 77340*

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**Learning Overview:** After attending this presentation, attendees will understand: (1) the potential for the use of a quantitative magnetic flux measuring device to differentiate between black-and-white toner-printed documents from different sources, and (2) the magnetic characteristics exhibited by toner printed documents and how to employ magnetic flux measurement techniques during comparative examinations between questioned and reference printed texts.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a method for quickly screening black-and-white toner-printed documents, reducing the need for more time-consuming or destructive methodology.

This study expands on questions raised by previous research into magnetic properties of toner. Specifically, this research investigates the effects of the external biasing unit on the repeatability of magnetic flux measurements of toner-printed documents. The hypotheses advanced were that: (1) the sample does not reach saturation magnetization for all particles if the sample distribution is abnormally shaped and the surface area of the toner exposed to the induction current is less than one-fourth of the total sample surface area, and (2) hysteresis effects can enhance or suppress the native magnetic flux of the sample depending on the sensor orientation relative to the native flux field of the sample. Both phenomena could lead to aberrant measurement values which are not representative of the true value for the sample and can increase the apparent sample inhomogeneity and measurement uncertainty if not controlled.

To test these hypotheses, measurements were conducted on five samples collected from different printing devices. The samples were printed with controlled grids of square, rectangular, and elongated rectangular blocks that all had the same area as well as line grids of differing densities. Thirty measurements were conducted on each sample and on each grid type, with the sensor oriented so the rectangles or lines were parallel to the induction current. The measurements were repeated in planes of rotation of 90°, 180°, and 270° counter to this original position. The values obtained for each sample in the different planes of rotation for the square grid were compared with a *t*-test to evaluate the presence and impact of hysteresis effects. The values obtained for each sample in the different planes of rotation for the rectangular grids were compared to those obtained for the square grids using the *t*-test to determine the impact of the induction current orientation effects.

It was found that both hysteresis and induction current orientation effects had a significant effect on the results obtained when comparing the measurements taken from one sensor orientation to those taken from another orientation (*p* value <.01). However, the magnitude of the hysteresis effects was smaller (<20% enhancement or suppression) than the magnitude of the induction orientation effects (up to 90% suppression). The hysteresis effects were also found to be reciprocal, with enhancement values mirrored by equal and opposite suppression values in another plane of orientation. This indicates that the hysteresis effects can be controlled via sampling methodology. New quantitative methods are needed to anticipate and correct for orientation-induced variation in the measured magnetic flux. Important inputs for such methods will include the surface area of the toner and its orientation relative to the induction current.

These findings have identified orientation effects as a significant source of variability in magnetic flux data and the need for new quantitative models that correct for this variability. The ability to recognize and correct for orientation-induced variations will be critical to the future implementation of a method using magnetic flux measurements in forensic laboratory settings, where there is a need for rapid, non-destructive screening of toner-printed documents.

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#### Questioned Documents, Toner, Magnetic Flux



### J16 Contested Estates: A Bloody Mess

*Khody R. Detwiler, BS\*, Lesnevich & Detwiler, Roaring Spring, PA 16673*

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**Learning Overview:** After attending this presentation, attendees will better understand how blood differs from writing ink formulas within the scope of the forensic document examination field, and why many certain writing production methods and instrumentation are simply not compatible with materials such as blood.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) showing the intricacies of conducting an analysis of extended handwritten entries produced entirely in blood, and (2) providing a unique perspective into the limitations that blood may impose upon certain production methods and writing instruments.

From examining letters and notes in homicide cases to identifying authors of forged checks, Forensic Document Examiners (FDEs) are often called upon to provide opinions in a variety of complex situations. Many of these cases unfold through a unique web of interesting facts and circumstances. Some of the most captivating cases seen in the forensic document examination field arise when family members become embroiled over money and assets in highly contentious estate matters. This presentation focuses on one such case involving the authenticity of a handwritten codicil, which determined the true heirs of a multi-million dollar estate. Although this may have seemed like a straightforward forensic handwriting case, it was anything but, as this codicil was written entirely in blood.

To make matters even more interesting, opposing counsel took the position that if the writing was deemed genuine, then the codicil was nothing more than a fabrication produced with the aid of an autopen or some other similar mechanical writing instrument. In addition to completing a forensic handwriting analysis to determine the authenticity of the writing at issue, counsel's position required further research to be conducted into identifying how or why certain methods of production would, or would not, be plausible due to the writing at issue having been produced with blood. This stage of the analysis revealed quickly that very little information is available within the scientific community pertaining to the examination of writings produced with blood.

This presentation will walk attendees through the entire examination and testimony process, highlighting the specifics of the actual analysis and research conducted, and will further discuss how the evidence was presented at trial, leading to a successful outcome.

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#### **Handwriting, Blood, Fountain Pen**



### J17 A Comparative Study of Generation Y and Generation Z Signatures

Nina A. Harnarine, BSc\*, Forensic Examiners, Inc, Toronto, ON M4W 3H1, CANADA

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**Learning Overview:** After attending this presentation, attendees will understand the handwriting characteristics observed in Generation Y and Generation Z signatures.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by discussing handwriting characteristics observed in the younger generations' signatures, those that are shared within Generations Y and Z, and if differences exist between the generations.

Before 1980, writing was taught in North American schools using copybooks from published writing systems. After 1980, there was a shift in the North American public schools for students to copy their teacher's writing on the chalkboard.<sup>1</sup> The North American educational curriculum over the years has had less focus on penmanship and writing instruction. As of 2006, some Provinces in Canada have eliminated cursive writing as a learning expectation in the curriculum.<sup>2</sup> As of 2013, approximately 45 states in the United States have removed cursive writing instruction from the curriculum.<sup>2</sup>

Generation Y or the Millennial Generation was born between the early 1980s and the mid-1990s. Generation Y was one of the first generations to grow up with computers, the internet, and cell phones. They were exposed to technology and many became technologically savvy. Several individuals from Generation Y were exposed to cursive writing instruction but have reverted to hand printing.

Generation Z or the Post-Millennial Generation was born between the mid-1990s and the mid-2000s. At a very young age, Generation Z was exposed to technologies, such as computers and tablets. They have become highly proficient in the use of technology. Some individuals within the younger generation have had no formal cursive writing instruction and are unable to write or even read cursive writing.

The younger generations often prefer to type on an electronic device versus writing with pen and paper. Speech-to-text is also a popular option. Video chat, text, or direct messaging is favored over handwritten correspondence.

This project sets out to compare the signatures of Generation Y and Generation Z. Participants from this study ranged in age from 18 to 39 years (born 1980 to 2001) and learned to write in North America. The Generation Y participants ranged in age from 24 to 39 years (born 1980 to 1995). The Generation Z participants ranged in age from 18 to 23 years (born 1996 to 2001). Ten specimen signatures were collected from each participant. Handwriting features that were assessed include: type of writing, style of writing, skill, case of writing, letter construction, connections, punctuation, initial and terminal strokes, size, flourishes, line quality, alignment, positioning, and range of variation.

It is hypothesized that Generation Z writers will have less connections, introduce more hand printed forms, have poorer line quality, and have a lower skill than Generation Y.

Signatures are important in authenticating documents. Do Generation Y and Z still value pen-and-paper signatures? The educational and societal changes that distinguish Generations Y and Z from past generations include: the lack of formal penmanship training in cursive writing and the shift toward electronic communication. Are there unique features in Generations Y and Z signatures that set them apart?

**Reference(s):**

1. Jan Seaman Kelly and Brian S. Lindblom, *Scientific Examination of Questioned Documents, Second Edition* (Boca Raton: CRC Press Taylor and Francis Group, 2006), 59-60.
2. Lisa Van De Geyn. The End of Cursive Writing in Schools? *Today's Parent*, November 26, 2013, <https://www.todayparent.com/family/cursive-writing-in-schools/>.

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**Signatures, Generation Y, Generation Z**



### J18 A Social Science Paradigm for Forensic Handwriting Identification

Carole E. Chaski, PhD\*, ALIAS Technology, LLC, Georgetown, DE 19947

**Learning Overview:** After attending this presentation, attendees will be able to evaluate the possibility of applying a social science paradigm to forensic handwriting identification using the model presented.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing: (1) principles for applying the social science research paradigm to forensic handwriting identification and other pattern-recognition forensic sciences, and (2) ways that this paradigm could shift current practices.

Forensic handwriting identification is practiced within governmental crime laboratories and independent consultancies and was the first forensic science to come under the scrutiny of the *Daubert* factors for scientific evidence.<sup>1</sup> In the past 20 years, there have been *Daubert* challenges, generally with mixed results: some exclusions, some inclusions, and some restricted inclusions of testimony.<sup>2,3</sup> The forensic document examination community, the legal community, and the academic research community have offered multiple solutions for meeting the *Daubert* factors, including the development of software for fully automated handwriting identification, such as CEDAR-FOX and FLASH-ID, proficiency experiments, and consensus-based standards in groups such as Technical (Scientific) Working Group on Questioned Documents and the Organization of Scientific Area Committees subcommittee for forensic document examination.<sup>4,5</sup> All of these solutions stem from the desire to provide forensic document examination, and especially handwriting identification, with an acceptable and feasible scientific foundation, objectivity, and mitigation of confirmation bias.

This presentation focuses on a social science research paradigm that can be applied to handwriting identification as well as other pattern recognition techniques. This social science paradigm is already accepted within major social sciences (e.g., psychology, linguistics, sociology).<sup>6-9</sup> While social sciences (e.g., psychology, linguistics, sociology) are known for strong quantitative analysis and laboratory experimentation, they also provide research guidelines for qualitative assessment based on educated judgments. The main purpose of this paradigm within the social sciences is to produce objective results when the analysis is based on qualitative assessment. This paradigm involves an administrator and a small team of at least three experts. The experts work independently (“blindly”) using a checklist as well as unstructured notes for the analysis of items, performing a qualitative assessment of the data. This paradigm provides a measure of reliability for the assessment using well-known and standard statistical procedures for inter-rater reliability (i.e., agreement among qualitative assessments).<sup>10</sup> The paradigm’s experimental design or workflow can easily be replicated. Finally, this paradigm can be adopted in both governmental laboratories and independent consultancies, which could lead to standardization in the field.

The social science paradigm has already been employed in forensic linguistics for the qualitative analysis of suicide notes and threat letters.<sup>11</sup> The workflow for this paradigm in forensic linguistics has been implemented in software to insure that: (1) the administrator manages the team objectively, (2) the team members actually do work independently (“blindly”) and cannot be influenced by each other’s assessment (thus avoiding group-induced confirmation bias), and (3) the administrator runs the automated quantitative assessment of the team’s collective decision and inter-rater reliability. The report based on this paradigm includes both the collective decision and the inter-rater reliability so that the strength of the decision can be evaluated using statistical information.

This social science paradigm differs in several important ways from current practices in both governmental crime laboratories and independent consultancies. Thus, there may be cultural resistance to adopting this paradigm. On the other hand, if adopted by the forensic document community, forensic handwriting identification could align with standard social sciences.

#### Reference(s):

1. *United States v. Starzeczyzel*, 880 F. Supp. 1027 (S.D.N.Y 1995).
2. Michael D. Risinger. Cases Involving the Reliability of Handwriting Identification Expertise Since the Decision in *Daubert*, 43 *Tulsa L. Rev.* 477 (2013). Available at: <http://digitalcommons.law.utulsa.edu/tlr/vol43/iss2/11>.
3. Zlotnick J., Lin J.R. Handwriting Evidence in Federal Courts—From *Frye* to *Kumho*. *Forensic Sci Rev.* 2001;13:87–99.
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5. Sita, J, Found, B, Rogers, D. K. Forensic Handwriting Examiners’ expertise for signature comparison. *J Forensic Sci*, Sept. 2002, Vol. 47, No. 5:1117-24
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7. Armstrong, D., Gosling, A., Weinman, J., and Mateau, T. The Place of Inter-Rater Reliability in Qualitative Research: An Empirical Study.” *Sociology*. 31(3) 597-606.
8. Milroy, L. 1987. *Observing & Analyzing Natural Language*. New York: Blackwell.
9. Richardson, J.T.E. (ed). 1996. *Handbook of Qualitative Research Methods for Psychology and the Social Sciences*. New York: Wiley-Blackwell.
10. Hallgren, K.A. 2012. Computing Inter-Rater Reliability for Observational Data: An Overview and Tutorial. *Tutor Quant Methods Psychol.* 8(1): 23-34. Available online at <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3402032/pdf/nihms372951.pdf>.
11. Chaski, C.E. and Huddle, D. Is This a Real Suicide Note? Authentication Using Statistical Classifiers and Computational Linguistics. *Proceedings of the American Academy of Forensic Sciences, 63rd Annual Scientific Meeting, Chicago, IL. 2011.*

#### Forensic Handwriting Identification, Social Science, Inter-Rater Reliability



### J19 Seen and Unseen Evidence of the Shroud of Turin and Use of the VP-8 Image Analyzer

*Janis Winchester, MS\*, Fort Myers, FL*

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**Learning Overview:** After attending this presentation, attendees will understand the Shroud of Turin and the use of the scientific instrument, the VP-8 Image Analyzer, that assists with demonstrating the apparent 3D-relief image on the Shroud of Turin.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining the Shroud of Turin and the methods and materials used with the VP-8 Image Analyzer to show the apparent 3D-relief image on the XYZ axis as the brightness information of the grayscale.

The Shroud of Turin documents an image of a man consistent with the events leading up to the crucifixion and death of Jesus of Nazareth, approximately two thousand years ago. It is believed to be the burial cloth which wrapped Jesus Christ when he was laid in the tomb, after he was taken down from the cross. Of interest today is that the linen cloth documents these historical events. The bodily image on the cloth indicates that the body was placed supine on half of the approximately 14-foot linen fabric, and the other half was pulled over the head, then laid on top of the body. Today this linen cloth is maintained in Turin, Italy, in a specially made environmental case.

Although the VP-8 Image Analyzer is no longer in production, other technologies may show the apparent 3D-relief image. The VP-8 provided one of the early analog image projections in the 1970s. This was of interest to the distinguished scientists, representing a vast range of scientific expertise, who were involved with the initial Shroud of Turin Research Project (STURP). The use of the VP-8 Image Analyzer was one of the deciding factors used by the scientists prior to their traveling to Turin, Italy, to examine the original linen cloth in 1978. The scientists examined the fabric, performed various tests and experiments focusing on what may have caused the image formation, and noted the other evidence they found on the cloth. They issued a scientific report of their findings in 1981 and described additional areas of research. The most comprehensive data on the Shroud of Turin is available on the website at [www.shroud.com](http://www.shroud.com). It is available to scientists and anyone interested in learning more about this unique mystery.

The VP-8 uses the input of a 2D photograph of the Shroud of Turin, from an image analysis camera, to visualize an apparent 3D relief image. What appears is that of a relief image of the Man of the Shroud, not seen on the normal 2D photograph. This unique phenomenon may indicate radiating energy, possibly photons from a possible blast of radiation parallel to gravity. The VP-8 records the data that is then imaged on the display monitor.

Given the uniqueness viewed on the display monitor of the possible photons from a potential source such as radiation, the VP-8 Image Analyzer assists in this visualization of the photographic grayscale. This characteristic, along with the fact that the image of the man of the Shroud of Turin is found on the inside of the folded linen, indicates the radiating energy came from the body of the man within the folded cloth. Additional research is ongoing.

This case study describes a unique phenomenon on the linen of the Shroud of Turin along with references for further investigations. Additional research, such as the examination for possible letter forms, pollen, body fluids, and other areas for forensic research is intriguing, along with how the image was formed on the burial cloth of the Shroud of Turin.

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#### **Shroud of Turin, VP-8 Image Analyzer, Evidence**



### **J20 The Discrimination of Primary and Secondary Impressions on the Second Page of a Two-Part Carbonless Copy Paper Using VSC40: A Case Study**

*Muneeb H. Khan, MPhil, Punjab Forensic Science Agency, Lahore, Punjab 54700, PAKISTAN; Khurram W. Mahmood, MPhil, Punjab Forensic Science Agency, Lahore, Punjab 53700, PAKISTAN; Muhammad Irfan Ashiq, PhD\*, Punjab Forensic Science Agency, Lahore, Punjab 54500, PAKISTAN; Mohammad A. Tahir, PhD, Punjab Forensic Science Agency, Lahore, Punjab, PAKISTAN*

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**Learning Overview:** After attending this presentation, attendees will be aware of the importance of performing examinations on an original questioned document instead of its photocopy and the need for the examination of all areas of the document instead of examining only a requested area of the document. A better understanding among the partner departments and agencies will be recognized with the goal of facilitating evidence-based justice.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a reference for the examination of questioned items beyond the scope of requested examinations.

In Pakistani banks, customers are required to fill out bank-issued, two-part, carbonless copy paper “Bank Deposit Slips” to deposit cash in their bank accounts. The depositor submits filled slips to the bank officer along with the cash deposit. The bank officer then verifies details and mentions the total cash amount received along with the number and denomination of bills. After all verifications, the bank officer cross-signs and dates the “Bank Deposit Slip” and returns the 2<sup>nd</sup> page (i.e., Depositor’s Copy) to the depositor. In normal practice, each set of “Bank Deposit Slips” is not placed as a separate set but in the form of booklet. This case study involves one such instance in which the depositor used a “Bank Deposit Slip” to deposit a cash amount of PKR 849,500 to the account of a seller for the purchase of some goods. However, he did not realize that it already contained secondary impressions on the 2<sup>nd</sup> page due to the pen pressure used on the previously filled “Bank Deposit Slip” in the booklet. Later, the seller registered a complaint with the local police station that the depositor has submitted a copy of a deposit slip after altering the amount from PKR 849,500 to PKR 8,049,500 and received goods against the altered amount. The case was submitted to the Questioned Document Department for analysis to examine suspected tampering on the questioned copy of the deposit slip.

To analyze the evidence for tampering, an examination was conducted utilizing the Foster & Freeman Video Spectral Comparator 40 (VSC40) and all fields on the questioned depositor’s copy of the “Bank Deposit Slip,” including the account title, account number, date, total amount in words, amount, total amount, depositor’s signature, and contact number, were examined. Denominations, quantity, and amount were examined by using the spot fluorescence filter in the range of 560nm–680 nm with a long-pass barrier filter 725nm in front of the camera of the VSC40. This report discusses quite unusual findings from this optical examination. Multiple primary and secondary impressions were deciphered. Based on the observations recorded by the use of the above-mentioned settings on VSC40, if only the requested fields were tested, it would have resulted in the false identification of tampering in the fields account title, account number, date, total amount in words, amount, total amount, depositor’s signature, and contact number. Complete examination of the questioned “Bank Deposit Slip” along with the firsthand knowledge of common banking procedures for depositing cash into an account in Pakistan, helped reveal the fact that the questioned “Bank Deposit Slip” did not contain any indications of tampering, but only contained the secondary impressions due to the previously placed two-part carbonless copy paper above it.

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#### **Video Spectral Comparator, Carbon-Less Copy, Primary and Secondary Impressions**



### J21 Contemporary Challenges, Expert’s Insights, and Judicial Perspectives on Forensic Document Examination Testimony

*Jan Seaman Kelly, BA\*, Forensic Dynamics LLC, Las Vegas, NV 89147; Stephanie Domitrovich, JD, PhD\*, Sixth Judicial District of PA, Erie, PA 16501*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the challenges encountered in providing testimony in a *Daubert* or *Frye* hearing and the perspectives of the judiciary who oversee the hearings.

**Impact on the Forensic Science Community:** The presentation will impact the forensic science community by showing the challenges faced in contemporary *Daubert* or *Frye* hearings as well as understanding the perspectives of the judiciary.

Prior to the 1993 decision in *Daubert vs. Merrill Dow Pharmaceuticals*, state and federal courts heard challenges to expert testimony relying on Federal Rules of Evidence 702 or *Frye v. United States*.<sup>1,2</sup> The Federal Rules of Evidence 702 states an expert who possesses the knowledge, skill, experience, training, or education may testify in the form of an opinion if it will assist the trier-of-fact. In *Frye v. United States*, the admissibility of scientific evidence is based on general acceptance.<sup>1</sup> Seventy years later, *Daubert v. Merrell Dow Pharmaceuticals* stated the judge, as the “gatekeeper,” is responsible in determining whether the scientific evidence the expert will testify to meets the five *Daubert* criteria.<sup>2</sup> The five criteria that are to be applied to determine if the expertise meets scientific rigor are as follows: (1) whether a method can or has been tested, (2) the known or potential rate of error, (3) whether the methods have been subjected to peer review, (4) whether there are standards controlling the technique’s operation, and (5) the general acceptance of the method within the relevant community. The *Daubert* Court also emphasized that these five factors be applied by trial judges in a flexible manner. Trial judges have great discretion in deciding on the admissibility of evidence; appeals from *Daubert* rulings are subject to a very narrow abuse-of-discretion standard of review.

Moreover, Federal Rule of Evidence 702 requires the following for expert testimony: A witness who is qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise if: (1) the expert’s scientific, technical, or other specialized knowledge will help the trier-of-fact to understand the evidence or to determine a fact in issue; (2) the testimony is based on sufficient facts or data; (3) the testimony is the product of reliable principles and methods; and (4) the expert has reliably applied the principles and methods to the facts of the case.

Also, under Federal Rule of Evidence 403, the trial judge may exclude relevant evidence if its probative value is substantially outweighed by the danger of unfair prejudice, confusing the issues, misleading the jury, undue delay, wasting time, or needlessly presenting cumulative evidence. Federal case law recognizes that certain situations call for exclusion of evidence that is of unquestioned relevance. These situations entail risks ranging from inducing decisions on purely emotional bases to confusing or misleading jurors. Trial judges must balance the probative value of and need for this evidence against the harm likely to result from admitting said evidence.

Several state courts, such as New York and Pennsylvania, still apply the *Frye* standard instead of the *Daubert* standards. Trial judges in *Frye* jurisdictions, in essence, defer to the scientists and admit expert opinion based on scientific techniques only where the techniques are generally accepted as reliable in the relevant scientific community.

Forensic document examiners have offered testimony in *Daubert* and *Frye* challenges for more than 20 years. A few of those challenges resulted in limited testimony or exclusion based on the gatekeeper’s determination that the profession lacked published standards and testing to establish reliability. As a result, the forensic document profession responded by drafting and publishing standards for various examinations, training, and conclusions, as well as participating in research studies conducted by academia.

This presentation will discuss the impact of testimony describing the profession’s work in publishing standards and the results of testing on the *Daubert* and *Frye* challenges that have occurred over the past several years in numerous state and federal jurisdictions. After attending this presentation, attendees will have a better comprehension of the trial court’s admissibility standards regarding expert testimony as to forensic document examination.

The importance of explaining in detail the basis for the issued conclusions, the established reliability of the field of forensic document examination as well as the practitioner through testing and research, and the work of the Organization of Scientific Area Committees (OSAC) subcommittee in drafting or revising current standards in the field of forensic document examination will all be discussed.

#### Reference(s):

1. *Daubert v. Merrell Dow Pharmaceuticals*. US Supreme Court 509.U.S.579,113S.Ct.2786, 125L. Ed.2d 469. 1993.
2. *Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923).

#### *Daubert*, Admissibility, Experts



### J22 Photo-Polymer Stamps: Their Examination and Identification

*F.L. Jim Lee, Jr., MS\*, Summit Forensic Document Examination Lab, Eden, UT 84310*

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**Learning Overview:** The goal of this presentation is to introduce attendees to the examination of defects in a stamp impression made from a photo-polymer stamp for the purpose of identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing awareness of the forensic document examination of photo-polymer stamps that the questioned document examiner may encounter.

Questioned document examiners may encounter diversity in the makeup of the stamps in this class of devices. “The diversity is not limited to classification based upon makeup. The actual material from which the stamp is constructed, and its mode of manufacture adds to the possible verities.”<sup>1</sup> A forensic document examiner must not only be aware of the many factors that can cause individual or unique features in a photo-polymer stamp and its impression, but also must be able to evaluate and interpret the significance of any specific characteristic and the relevance it may have to the question or problem under examination. The Scientific Working Group for Forensic Document Examination (SWGDOC) has produced standards that supersede the *ASTM E 2289-03 Standards*, published by the American Society for Testing and Materials, International. These published standards provide the procedures that should be used by forensic document examiners when conducting examinations and comparisons involving rubber stamps and their impressions. These standards are of great importance to the questioned document examiner for it is the questioned document examiner who must be “able to interpret the significance of any particular feature, the relevance it has to the problem in hand, the weight of opinion it merits and the depth of knowledge that can be gained from it.”<sup>2</sup> Of consideration to the forensic document examiner should always be the possibility of the existence of a duplicate stamp. Because of the existence of this possibility, often the forensic document examiner should maintain a conservative approach when rendering his or her opinion on examinations. Oftentimes, an inconclusive finding will need to be rendered instead of a positive identification of a specific stamp.

This presentation will provide an overview of the process involved in the manufacture of photo-polymer stamps. Additionally, this presentation will discuss the results of research conducted by the presenter in evaluating the results of the attempt of several forensic document examiners to examine and compare stamped impressions from a specific photo-polymer stamp manufactured for this purpose and the accurateness of their opinions in comparison with the ground truth solution of the problem provided.

**Reference(s):**

- <sup>1</sup> Hilton, Ordway. *Scientific Examination of Questioned Documents*, rev. ed., Elsevier Science Publishing Co., New York, 1972, p. 72.
- <sup>2</sup> Herkt, A. Rubber Stamps. Manufacture and Identification. *Journal of Forensic Science Society*, Volume 25, Issue 1, January 1995), pp. 23-38.

**Rubber Stamps, Photo Polymer, Manufacturer**





### J23 Invisible Migration of Luminescent Components in Inks in Crossed-Line Intersections

*Rhett Williamson, PhD, Florida International University, Miami Beach, FL; Daniela Djidrovaska, MS, Interpol Lyon, Lyon, FRANCE; Andrea Ledic, BS\*, Croatian Forensic Science Centre "Ivan Vucetic," Zagreb, Europa 10000, CROATIA; Vesna Antikj, MS, Forensic Department, Skopje, MACEDONIA; Stjepan Brzica, BS, Forensic Science Centre "Ivan Vucetic," Zagreb, CROATIA; Rolf Hofer, PhD, Forensic Science Institute Zurich, Zurich, SWITZERLAND; Jose R. Almirall, PhD, Florida International University, Miami, FL 33199*

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the interactions of luminescent inks in crossed-line intersections.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping determine the chemical identity of the luminescent compounds present in the formulation of inks to better understand the interaction of different inks in crossed-line intersections.

Characterizing the fluorescent components of inks, most commonly dyes, is very useful in document examination for association, discrimination, and intelligence purposes. Optical methods such as fluorescence, infrared luminescence, and spectroscopic methods such as Fourier Transform Infrared (FTIR) spectroscopy, and Ultraviolet/Visible (UV/VIS) spectroscopy rely on these properties. In addition, chromatographic, spectroscopic and mass spectrometric techniques such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), paper chromatography, Nuclear Magnetic Resonance (NMR) spectroscopy, and Liquid Chromatography/Mass Spectrometry (LC/MS), and Gas Chromatography/Mass Spectrometry (GC/MS) have been applied to the analysis of dyes and pigments.

In recent years, laser desorption has been applied to the analysis of inks, with success in characterizing the dye, pigment, and, more limitedly, the polymeric content of the inks on paper in combination with other techniques such as Thin-Layer Chromatography (TLC) and Raman spectroscopy.

This study involved independent analyses of several inks by three laboratories in Croatia, Macedonia, and the United States. A combination of TLC, GC/MS, HPLC, Matrix-Assisted Laser Desorption Mass Spectrometry (MALDI-MS), Direct Analysis in Real-Time Mass Spectrometry (DART<sup>®</sup>-MS), and LC/MS were implemented by the three laboratories in order to characterize the luminescent components of inks present in crossed-line intersections. A combination of luminescent compounds, including crystal violet and methyl violet, were characterized and identified to be present in mixtures in the ink formulations utilizing each of the analytical techniques included in this study. When inks interact in crossed-line intersections, invisible migration may occur. This migration is a result of the intersection of the liquid inks with one another, causing a solvation effect. This causes the luminescent compounds, in this case crystal violet and derivatives. However, the temporal sequence of deposition of inks present in crossed-line intersections could not be determined. The protocol described here allows for the isolation and characterization of luminescent compounds present in the formulation of inks to varying degrees, and the information presented here can be used in the future establishment of a standard protocol for the identification of luminescent compounds in inks.

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#### **Ink, Crossing-Line Intersection, Crystal Violet**



### J24 Reasons for Qualified Opinions in Forensic Document Examination

Jane A. Lewis, MFS\*, Milwaukee, WI 53202

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**Learning Overview:** After attending this presentation, attendees will be familiar with the current reasons for qualified opinions in forensic document examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by developing an appreciation for the major causes of less-than-definite findings in the reports of forensic document examiners resulting from their casework examinations of disputed handwriting.

This study was motivated by recent questions from attorneys asking why less-than-conclusive opinions were necessary in reports. When submitters deliver questioned handwriting evidence for analysis, they expect that the resulting opinion will be an identification or an elimination. The use of electronically saved documents at low resolution and the tendency of individuals and businesses to scan and destroy original documents imposes limitations on the examinations of forensic document examiners. This study was conducted to determine the main reasons for questioned handwriting cases resulting in qualified opinions. Scales of opinions are typically used by forensic document examiners to convey the level of confidence that the evidence in the case will support. The goal of each examination is to determine the origin or authenticity of the questioned material. Limitations of the evidence will lead to a less-than-definite conclusion expressed as a qualified opinion. Appreciation for the reasons behind qualified opinions will help spur attorneys and investigators to search for the best quality case evidence. Original questioned and known documents will provide the most detailed information to allow document examiners to properly do their work.

Case files from 2015–2017 and the first half of 2018 were reviewed to determine the reasons for qualified opinions in each case. Ten specific reasons for qualified opinions were discovered from studying 49 cases. Evaluation of each case and the reasons for the qualified opinions generated were tallied. The three main reasons for qualified opinions in the case files were: (1) the lack of contemporaneous known writing, (2) the lack of known writing comparable to the questioned writing, and (3) the submission of poor clarity copies. The submission of poor clarity copies was the most common reason for a qualified opinion in a questioned handwriting case. A substantial number of cases displayed two reasons for reaching a qualified opinion.

The study sought to specify the precise reasons that forensic document examiners rendered qualified opinions in questioned handwriting cases. The study pointed to three significant reasons and seven other important reasons for the findings. The study suggests that informing submitters of the specific reasons for qualified opinions in questioned handwriting cases will lead investigators and attorneys to strive for the best evidence submissions in each case, which will include original questioned and known documents or good quality first-generation copies.

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#### Handwriting, Qualified Opinion, Copies



### J25 The Admissibility of Hand Printing Identification in the United States Courts

Karen J. Nobles, BA\*, Forensic Document Services, LLC, Pensacola, FL 32591-0411

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the admissibility of hand printing identification in the courts in the United States.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information and tools to improve the success rate of hand printing testimony.

Both federal and state courts in the United States have generally treated expert testimony from a forensic document examiner about the identification of handwriting and the identification of hand printing in the same manner. Logically, this seems to be the correct approach because the methodology for the comparison of questioned and known handwriting samples and the comparison of questioned and known hand printing samples is the same. In fact, the *Standard for Examination of Handwritten Items*, published by the Scientific Working Group for Forensic Document Examination (SWGDOC) in 2015 defined the type of writing covered by the standard as, “hand printing, cursive writing, numerals, symbols, or combinations thereof ....” In addition, most of the historical and current literature that discusses handwriting identification does not differentiate between cursive handwriting and hand printing when discussing the methods and procedures for examination and comparison. However, in some legal cases, the court has addressed hand printing identification separately from handwriting identification. In those courts where the identification of hand printing has been evaluated individually, hand printing identification has been subjected to numerous inconsistent decisions. Expert testimony about hand printing identification has been randomly admitted, excluded, and limited in court districts across the United States. After conducting both legal and non-legal research and reading through all the publications and the opinions in the relevant cases, there is a notable anomaly in the decisions regarding hand printing evidence in the United States courts. If expert hand printing testimony is proffered, the court generally admits the testimony for the trier-of-fact to consider in making their decision. In fact, prior to *Daubert*, research for this study was unable to find a single instance in which hand printing testimony was excluded. However, post-*Daubert*, if the admission of the hand printing testimony is challenged, the testimony of the handwriting expert is generally limited, or even excluded. If a challenge is not filed, the handwriting expert can testify to the identification of hand printing as routinely as any other examination.

This study looks at the reasoning of the court in each of these cases, especially considering the criteria developed in the landmark case of *Daubert v. Merrell Dow Pharmaceuticals*, and provides both historical and current supplemental publications and legal decisions that can be used by forensic document examiners to support the reliability of hand printing identification.<sup>1</sup> This supplemental information can be utilized to satisfy the court’s requirements, whether it applies the *Frye v. United States of America* standard (general acceptance) or the *Daubert* standard, in which the judge is tasked with determining if the science meets each of the five criteria.<sup>2</sup>

**Reference(s):**

<sup>1.</sup> *Daubert v. Merrell Dow Pharmaceuticals*. US Supreme Court 509.U.S.579,113S.Ct.2786, 125L. Ed.2d 469. 1993.

<sup>2.</sup> *Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923).

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**Hand Printing, Admissibility, Expert Testimony**



### J26 Pairwise Comparison Scores for Handwritten Questioned Documents

Danica Ommen, PhD\*, Iowa State University Department of Statistics, Ames, IA 50011; Cami Fuglsby, MS, Department of Mathematics and Statistics, Brookings, SD 57007; Christopher P. Saunders, PhD, Brookings, SD 57006; Michael Caligiuri, PhD, University of California, San Diego, CA 92093; Linton Mohammed, PhD, Forensic Science Consultants, Inc, Burlingame, CA 94010-2017; JoAnn Buscaglia, PhD, FBI Laboratory, CFSRU, Quantico, VA 22135

**Learning Overview:** After attending this presentation, attendees will better understand the value of pairwise comparison methods for designing tests of scientific validity for forensic feature comparison methods.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating an alternative use for pairwise comparison methods beyond automated source identification applications.

**Hypothesis Statement:** Methods resulting in pairwise comparison scores can be used in designing tests of scientific validity for a variety of forensic feature comparison methods, specifically in the case of validating questioned document examiners' conclusions.

In 2016, the President's Council of Advisors on Science and Technology released a report that advocated for further research into the scientific validity of all forensic feature comparison methods, including methods for handwriting evidence.<sup>1</sup> One of the research initiatives identified by the Organization of Scientific Area Committees, overseen by the National Institute of Standards and Technology, in response to this report seeks to validate the conclusions expressed by forensic document examiners regarding the weight or the value of the evidence in a particular comparison. To design the experiments needed to test examiners' conclusions, it is often necessary to pair handwritten documents with various levels of correspondence for the test comparisons performed by the examiners. In this way, the document examiners' conclusions will not be skewed by receiving, for example, only very easy comparisons. Since this pairing needs to be done quickly and effectively, we have been developing an automated method of comparing two documents that would produce a "score" indicating the level of similarity between two documents. Scores that are high indicate writing that is visually similar, and scores that are low indicate writing that is visually dissimilar. This score can then be used to provide the examiners with a range of difficulty in the test comparisons. The idea is that the person designing the test comparisons can pick out test comparisons that are from different writers with a high score to provide examiners with difficult exclusions, and test comparisons that are from the same writer with a low score to provide examiners with difficult inclusions. The test designer may also want to select easier test comparisons for both inclusions and exclusions, and a variety of pairs for comparison with moderate levels of difficulty.

In this presentation, a pairwise comparison score for images of scanned handwritten questioned documents using both open-source and proprietary automated image feature-detection methods will be developed. The Scale-Invariant Feature Transform (SIFT) and the Speeded-Up Robust Features (SURF) algorithms have demonstrated potential to identify important features of images for the purpose of comparing two general images of any content.<sup>2</sup> Woodard et al. demonstrated the use of these open-source algorithms for automated writer identification systems in the case of Arabic handwriting.<sup>3</sup> Gannon Technologies Group developed the proprietary FLASH ID algorithm to perform a similar writer identification task, regardless of the written language.<sup>4</sup> However, the algorithms first need to be trained on a set of images to create a dictionary/codebook of similar features to look for in the test images. Once the algorithm is trained, a single image is provided as the input, then a numerical representation of the important features in that image is output to the user. A method of comparing these numerical outputs to produce a similarity score indicating the level of correspondence between two scanned images of handwriting (cursive or print) will be demonstrated. The use of the scores on a collection of handwritten and handprinted English-language documents collected as part of an ongoing research effort will also be presented.

#### Reference(s):

1. President's Council of Advisors on Science and Technology. *Forensic Science in Criminal Courts: Ensuring Scientific Validity of Feature-Comparison Methods*. Washington, DC: Executive Office of The President's Council of Advisors on Science and Technology, <https://www.whitehouse.gov/administration/eop/ostp/pcast/docs-reports> (2016).
2. Lowe, D. (2004) Distinctive image features from scale-invariant keypoints. *International Journal of Computer Vision*, 60, pp. 91-110.
3. Woodard, J., Lancaster, M., Kundu, A., Ruiz, D., and Ryan, J. (2010). Writer Recognition of Arabic Text by Generative Local Features. *IEEE*, <https://ieeexplore.ieee.org/document/5634495>.
4. Gantz, D., Miller, J., and Walch, M. (2005). Multi-Language Handwriting Derived Biometric Identification. *Proceedings 2005 Symposium on Document Image Understanding Technology*, pp. 197-210.

#### Scores, Handwriting, Pairwise Comparisons



## Questioned Documents – 2019

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### J27 The Evolution of Security Features in International Travel Documents

*Carolyn Bayer-Broring, MFS\*, Immigration & Customs Enforcement, McLean, VA 20598-5116*

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**Learning Overview:** After attending this presentation, attendees will have a more thorough understanding of how the construction of international travel documents evolves to meet new standards and thwart counterfeiters.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a better understanding of the more common security elements and features that appear in international travel documents, showing how those elements evolve as needs arise.

For international travel, a person is usually required to provide documentary proof of citizenship for their home or adopted country before being allowed to board a train, boat, or plane, or to cross a country's border. In most cases, the required travel document is a passport or similar booklet that contains personalized data, which normally includes the date of birth and image of the bearer, to establish identity.

In the early 20<sup>th</sup> century, when passport use first came into the lexicon, the documents were not very sophisticated and were, in fact, sometimes very rudimentary. As their use became more widespread, the information contained in passports became more valuable and, thus, more susceptible to fraud and theft. Recognizing the valuable nature of that information, passport producers understood the need to secure the personalized data against misuse and counterfeiting. In earlier documents, that security was most often achieved using one or two security features of varying degrees of sophistication. As international travel became more popular, affordable, and more widely available, the documents evolved to become more sophisticated and secure.

In the 1940s, the International Civil Aviation Organization (ICAO) was formed by the United Nations to govern practices and standards regarding air navigation, including aspects such as flight inspection and facilitation of border-crossing procedures. As the ICAO developed and grew, among its many duties, it became the de facto dictating body for passport security. The ICAO was responsible for developing standards for the advancement and use of machine-readable travel documents, and more recently (in 2015) has developed standards for the design and use of biometric passports, which utilize Radio Frequency Identification (RFID) chips to store the valuable personalized data and information.

In this presentation, attendees will see a selection of international travel documents, showing how the documents evolved and advanced from the days prior to ICAO conformity through the progression of adding security features over the years to bring them into compliance. As travel documents continue to hold high value for criminals, such as terrorists and human smugglers, the necessity remains for passport designers to stay one step ahead of the counterfeiters. To meet these demands, document security designers are now developing more sophisticated and expensive features to thwart criminals. Savvy document designers recognize not only the need to utilize these sophisticated security features and safeguards, but to continue to meet financial demands that often limit how much can be spent on each booklet. This presentation will allow viewers to see how passport designers use a layered approach of security features of varying cost and sophistication to meet these goals.

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#### **International Travel Documents, Security Features, Counterfeiting**



### J28 Adding an Objective Approach to Questioned Document Examination Using Principal Component Analysis (PCA) and Mahalanobis Distance

Loren M. Williams, BS\*, Bethlehem, PA 18020; Lawrence Quarino, PhD, Cedar Crest College, Allentown, PA 18104; Morgan Mills, MSFS, Burlington, NC 27215

**Learning Overview:** After attending this presentation, attendees will better understand the potential role that statistical analysis can play in helping to support subjective determinations of authorship in questioned document examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating a simplified way to incorporate statistics into questioned document examination.

Document examiners have a skill that lay people do not. Studies have shown that professional examiners make fewer mistakes and are more accurate than laypeople when determining authorship of writing.<sup>1,2</sup> This skill, which is based on experience and expertise, is essential in helping to determine fraudulent writing and to correctly assign the writing origin of documents.

However, after the release of the National Academy of Science's Report on strengthening forensic science in 2009, it was made clear that objective measures of comparison are needed to augment and strengthen conclusions that are essentially subjective in nature.<sup>3</sup> One possible approach is to couple the skill of questioned document examiners with statistics to create a more objective methodology, allowing stronger conclusions to be made. Since questioned documents offer measurable characteristics such as the height of letters, the ratio of heights of adjacent letters, and the distance between words, the potential to use some form of multivariate statistical analysis in questioned document examination exists. To this end, this study incorporated the use of PCA and Mahalanobis distance to classify groups of known writing. In addition, the potential of using this method to compare an unknown with exemplar writing was explored.

To test this, 20 handwriting samples were collected from 50 individuals. The handwriting samples were all the same and taken on four different days. Writing was in script and the template used was a bank check. Various known features, such as height of capital and lowercase letters, distances between words, and ratios of letter heights, were measured using Adobe® Photoshop® 2017. Measurements were placed into a spreadsheet and used to perform PCA to attempt discrimination of handwriting between individuals. Of the 50 individuals, 32 were able to be distinguished visually when examining the principal components in a 3D plot. Unknown writing samples taken from 10 participants in the original 50 were collected and compared to the dataset. Using PCA, it was determined that each unknown could be visually placed into the correct data set. In addition, Mahalanobis distances were also calculated. Results from this statistical test showed that 8 of the 10 unknowns were able to be correctly classified into their respective data sets. An additional 10 unknowns were collected from individuals not in the original group of 50 and were compared to the dataset. The visual analysis using PCA indicated 9 of the 10 unknowns were not classified into any of the original 50 datasets, although the Mahalanobis distances placed several of the unknowns into one of the original 50 datasets based on criteria used in the study.

Results from the study seem to indicate that PCA offers the potential of objectively confirming conclusions reached in traditional questioned document examinations. Going forward, results reached in this study should be confirmed by the subjective examination of a trained document examiner. If results in this manner are consistent with the statistical analysis, it will lend credence to the use of statistical analysis (PCA in particular) to be used in tandem with traditional handwriting examination. This could help alleviate the criticisms often levied on handwriting comparisons as being too reliant on subjective analysis. Finally, PCA could be a tremendous tool in showing the variability in handwriting exemplars taken from individuals. Objective determination of this variability can be helpful in assessing the validity of particular exemplars used in writing comparisons.

#### Reference(s):

1. Kam M., Fielding G., Conn R. Writer Identification by Professional Document Examiners. *J Forensic Sci* 1997;42:778–86.
2. Sita J., Found B., Rogers D. Forensic Handwriting Examiners' Expertise for Signature Comparison. *J Forensic Sci* 2002;47:1117–24.
3. National Research Council (NRC), Committee on Identifying the Needs of the Forensic Science Community. (2009). *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press. 1-328.

#### Principal Component Analysis, Mahalanobis Distance, Handwriting Comparisons



### J29 An Exploratory Analysis of Handwriting Features: Investigating Numeric Measurements of Writing That Are Important for Statistical Modeling

*Amy M. Crawford, MS\*, Center for Statistics and Applications in Forensic, Ames, IA 50011; Alicia L. Carriquiry, PhD, Center for Statistics and Applications in Forensic, Ames, IA 50011*

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**Learning Overview:** The goal of this presentation is to provide insights into which features of handwritten documents are important for statistical modeling with the task of writer identification and to discuss how these features overlap with features that questioned document examiners typically examine.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing ongoing forensic statistics research to practitioners in the field. Collaboration and communication is fundamental to the success of emerging research.

In recent years, researchers have begun to uncover the process that document examiners follow when conducting a visual analysis of documents. This has been done using several approaches. One of the most fascinating is eye movement tracking, where a device tracks where the eyes are drawn and how long they stay fixated at certain locations on writing samples. This provides insight into which features are seemingly important to examiners when making decisions of writership. There has also been a significant emphasis on the use of data and statistical methodologies to assist in comparison of handwritten documents. For us, data are numeric measurements extracted from handwriting that has been scanned and processed. This research conducts a statistical analysis of measurements that have the power to discriminate between writers and will be important to a statistical model.

Decomposing a document to focus on smaller, meaningful bits of writing at a time lends nicely to an algorithmic approach to writer identification. These pieces of writing, often corresponding to letters, are treated as small graphs with nodes and edges and collect measurements from the graphs. Measurements such as curvatures, lengths, and slants of edges, relative heights of graph nodes, the diameter and shapes of loops, and many more are taken into consideration.

An exploratory analysis with a multitude of measurements is conducted. This is done by comparing features of graphs with similar structures across writers to investigate their ability to discriminate between writers. Measurements that separate writers well in a numeric capacity will be useful for statistical models. Such models are designed to complete the task of writer identification. The results of this feature analysis are discussed with relation to features of writing that document examiners typically consider important in casework as well as results from eye-tracking studies.

Since writing is a realization of a physical action and one cannot expect measurements taken from a single writer to be identical for every repetition of writing, a within-writer analysis is conducted. The writing samples used as data for this analysis were collected from a variety of individuals across the country. Participants were asked to complete writing tasks on a few occasions, each one month apart. The results of the within-writer analysis with respect to samples taken from a single writer with the same content and differing content will be discussed. These comparisons are conducted for writings in a single day, one month apart, and two months apart. These statistical analyses will provide numerical insights into how variable writing is for a single writer over varying periods of time.

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#### Document Examination, Statistics, Writing Features



## Questioned Documents – 2019

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**J30**    **WITHDRAWN**





### J31 A Comparison of Gaze Behavior in Sequential Versus Simultaneous Presentation of Signatures in a Handwriting Comparison Task

Mara L. Merlino, PhD\*, Kentucky State University, Frankfort, KY 40601; Chandler D. Al Namer, BA, Kentucky State University, Frankfort, KY 40601; Derek L. Hammond, BA, U.S. Army Criminal Investigation Laboratory, Forest Park, GA 30297-5205; Robert S. Olson, Kentucky State University, Frankfort, KY 40601; Pierre Easley, BA, Kentucky State University, Frankfort, KY 40601

**Learning Overview:** After attending this presentation, attendees will understand some of the principles of cognitive psychology and the use of eye-tracking technology to study attention and feature-matching processes as they relate to the presentation of writing information in forensic document examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating different methods of unmasking information contained in writing samples on the visual behavior and decision accuracy in a signature comparison task.

A substantial body of research addresses the cognitive mechanisms involved in attention and visual search. Relational models of visual search demonstrate that visual attention can be guided by attending to specific feature values, such as color, size, or intensity, by inhibiting attention to irrelevant features, or by directing attention to how stimuli differ. Many current theories of attention propose that attention is based on “the interplay of a bottom-up, saliency-based attentional system and a top-down, feature-specific selection mechanism.”<sup>1</sup> According to Becker, another type of information that guides attention is relational information about the target, or information about how the irrelevant information of a non-target differs from the features of the target.<sup>1</sup> Relational models place the target in relation to its context, offering more specific (e.g., directional) information about differences.<sup>1</sup> This relational aspect of attention may also be influenced by the presentation formats of signature specimens.

Research on eyewitness testimony and studies on the use of sequential vs. simultaneous lineups in criminal investigations provide a conceptual framework for studying the effects of method of presentation on the deployment of attentional resources and the examination and weighting of signature specimens. Although simultaneous lineups are the most common procedure used in the United States, researchers argue that simultaneous lineups, which are relational in nature, result in biased judgments.<sup>2</sup> Witnesses are required to use “relative judgments” by comparing lineup members to each other, rather than relying on their memory for such comparisons. This method is problematic because if the true perpetrator is not in the lineup, then the witness will often implicate the lineup member who most closely resembles the perpetrator, resulting in a false identification.<sup>3</sup> Much of the laboratory research in this area indicates that sequential lineup procedures are less likely to be biased than are simultaneous lineups.

Subsequent field research using the double-blind sequential method of lineup presentation indicates that, in practice, the simultaneous procedure may produce fewer false identifications than the sequential procedure. The sequential lineup forces witnesses to resort to “absolute judgment” in which they can compare each photograph in the lineup only to their memories of the offender, but field studies of this phenomenon have demonstrated either equivocal or contrary results to lab studies.<sup>4</sup>

Kassin et al. stated, “Examiners should work ‘linear’ rather than ‘circular,’ thus initially examining the evidence from the crime scene and documenting their findings before making comparisons against a target. This will eliminate the potential influence of the target on how information is processed and the weight assigned to it.”<sup>5</sup>

The use of sequential rather than simultaneous presentation of signatures during a handwriting identification task has been discussed in the Forensic Document Examiner (FDE) community in Australia, but this procedure has not been empirically tested. This presentation reports the results of an empirical experiment investigating the differences in examination processes and the accuracy of signature comparison decisions when signatures are presented simultaneously or sequentially during a signature comparison task.

Sixteen signatures were presented during an experimental eye-tracking procedure (NIJ Award # 2015-DN-BX-K069). Signature comparisons were counterbalanced so that all 16 signature comparisons were viewed either simultaneously or sequentially. Participant gaze behavior was recorded using a Tobii X2-60 eye-tracking system. Results will be discussed in the context of current discussions about sequential unmasking of forensic evidence features.

#### Reference(s):

1. Becker, S.I. (2010). The Role of Target-Distractor Relationships in Guiding Attention and the Eyes in Visual Search. *Journal of Experimental Psychology: General*, 139(2), 247-265. <http://dx.doi.org/10.1037/a0018808>.
2. Wells, G.L., and Olson, E. (2003). Eyewitness Testimony. *Annual Review of Psychology*, 54, 277-295.
3. Wells, G.L., and Seelau, E. (1995). Eyewitness Identification: Psychological Research and Legal Policy on Lineups. *Psychology, Public Policy and Law*, 1, 765-791.
4. Mecklenburg, S.H. (2006a, March). *Report to the Legislature of the State of Illinois: The Illinois Pilot Program on Sequential Double-Blind Identification Procedures*. Illinois State Police, Springfield IL.
5. Kassin, S.M., Dror, I.E., and Kukucka, J. (2013). The Forensic Confirmation Bias: Problems, Perspectives, and Proposed Solutions. *Journal of Applied Research in Memory and Cognition*, 2, 42-52.

#### Decision Accuracy, Presentation Context, Presentation Order



### J32 Forensic Characterization and Discrimination of Manila Envelopes

*Maria I. Sanchez-Melo, MS\*, West Haven, CT 06516; Virginia M. Maxwell, DPhil, Department of Forensic Science, West Haven, CT 06516; Irina Geiman, MS, United States Secret Service, Washington, DC 20223; Brooke W. Kamrath, PhD, University of New Haven, West Haven, CT 06516*

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**Learning Overview:** After attending this presentation, attendees will understand the meaningful physical and chemical differences among manila envelopes produced by different manufacturers that can be used to discriminate and/or identify several brands of this type of envelope.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by evaluating commonly employed physical and chemical analytical techniques to determine which offers the greatest discrimination potential, thus developing an analytical protocol for the forensic analysis of manila envelopes.

Envelopes are important probative items of evidence that are analyzed by forensic document examiners as they can be found at crime scenes when ransom, threat letters, or potentially harmful substances are sent to victims in an envelope through the mail. The use of manila envelopes in the United States is quite common because they are made of thick and durable manila paper; thus, they are frequently used to transport or send documents. There does not exist previously published research on the physical or chemical characterization of manila envelopes. Although there are many studies on the forensic analysis of office paper and paper-based banknotes, and only a few on white envelopes, a gap in the research has been identified. The ability to offer a way to systematically analyze manila envelopes, starting with the non-destructive techniques and using the most discriminating techniques, will be useful for the document examiner and the investigation.

The purpose of this research was to analyze manila envelopes using analytical methods commonly used for the analysis of paper and adhesives to be able to characterize and compare those sold by different manufacturers as well as the envelopes included in the same and different batches from the same manufacturer. Thus, this study investigated whether there are meaningful differences among them, and if these can be used for forensic discrimination and/or identification of the manufacturer. Samples from five manila envelopes, size 9"x12", from three boxes purchased from ten different brands were analyzed to evaluate the commonality between brands and determine the best methods for the discrimination of the envelopes. The analytical methods studied in this research included physical measurements of the envelopes and their folds, color examination using a colorimeter, the use of Alternate Light Sources (ALS), and chemical analysis using Thin-Layer Chromatography (TLC), Attenuated Total Reflection/Fourier Transform Infrared (ATR/FTIR) Spectroscopy, Raman Spectroscopy, X-ray Fluorescence (XRF), and X-ray Powder Diffraction (XRD).

Results indicate that IR spectroscopic analysis of the manila paper did not show meaningful differences; however, it was able to discriminate the adhesive between several of the various brands. Significant differences between brands were observed based on Raman spectra of the manila paper, as well as several physical measurements, including weight and thickness. Thus, it was concluded that detectable chemical and physical differences in the paper and adhesives of manila envelopes can be used for brand discrimination and have the potential to be used for brand identification.

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#### **Manila Envelopes, Physical and Chemical Analysis, Brand Discrimination**



### J33 An Examination of the Influence of Various Parameters on the Quality of Laser Printer Printouts

*Jasna Galekovic\**, Forensic Science Centre Ivan Vucetic, Moi, Zagreb 10000, CROATIA

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**Learning Overview:** The goal of this presentation is to show attendees how variable print parameters of laser printers and various file formats can affect the print quality of laser printers.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness that print quality of laser printers, apart from other parameters such as substrates, replacement toners, etc., are greatly influenced by the variable parameters of the printer itself as well as the file format being printed.

Forensic document examiners are often asked to determine whether two or more laser-printed documents were produced by the same laser printer. The examination of questioned documents can be performed using multiple techniques, such as microscopic examination, physical examination, and chemical examination (Fourier Transform Infrared Spectroscopy (FTIR), X-ray microanalysis, etc.). The physical analysis includes examinations of toners (type, fusion methods, etc.), possible printer identifying characteristics (roller marks, repetitive defects marks, etc.), and printout quality.

The goal of this study was to determine in which way the variable printer's parameters, such as print resolution, color or black/white printing, etc., as well as different file format (.txt, .pdf, .jpg, .png), have influence on printout quality. In the present study, more than 200 printout samples were taken from five different color laser printers. The number of printed samples on each printer depended on the number of their predefined variable parameters. Several files were used for printing on each of the printers; one file was created in MS Word® (letters and numbers in Arial and Times New Roman font), then saved in different file formats (.txt file, high-compressed .pdf file, low-compressed .pdf file); another file was a .pdf file containing letters, numbers, graphics, vectors, and images. Standard office paper (A4, 80g/m<sup>2</sup>) was used for preparing these samples. The examinations of samples were performed using non-destructive methods, such as video-spectral and stereomicroscopic analysis.

This study has shown that changing the parameters of the printer itself and changing the file format that is printed on the same printer causes significant differences in print quality. For example, there is a significant difference in letter size and the shape of the same letters and numbers between the print samples of a file saved as a high-compressed PDF file and a file saved in plain TXT format. Furthermore, changing the printer resolution and other available parameters, such as color balance, edge control, halftone options, RGB graphics, etc., also have a great effect on the printout quality.

Based on the results obtained, it is possible to conclude that the document experts should know that the differences in the printing quality of two or more of the questioned documents do not necessarily mean that they are manufactured on different laser printers and that other available test methods are needed to provide more reliable conclusions.

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#### Laser Printers, Variable Parameters, Printout Quality



### **J34 Understanding the Impact of Human Factors on Forensic Examinations: Recommendations From the Expert Working Group for Human Factors in Handwriting Examination**

*Melissa K. Taylor, MA\*, Gaithersburg, MD 20899; Ted M. Burkes, BS\*, FBI Laboratory, Quantico, VA 22135; Emily J. Will, MA\*, Raleigh, NC 27658; Reinoud D. Stoel, PhD\*, Laan van Ypenburg 6, Den Haag, NETHERLANDS*

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**Learning Overview:** After attending this presentation, attendees will better understand: (1) the general themes of human factors and organizational theory, and (2) the findings and recommendations of the Expert Working Group on Human Factors in Handwriting Examination.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping forensic professionals understand the impact of human factors on the handwriting examination process.

Much has been written in recent years about the role of human factors in forensic science. Human factors research examines the interactions between humans and other elements of a system—technology, training, decisions, products, procedures, workspaces, and the overall environment—with the goal of improving both human and system performance. Human factors analysis can be used to advance our understanding of the true nature of errors in complex work settings such as crime laboratories. Research in this area has identified factors that contribute to inefficiencies and quantified the effects of human and organizational factors on the performance of critical tasks. Causes of error include fatigue, workload, cognitive overload, poor interpersonal communications, imperfect information processing, team mistakes, and flawed decision making. Error management requires an understanding of the nature and extent of error so that appropriate changes can be made to the conditions that induce error, actions that prevent or mitigate error can be employed, and personnel can be properly trained to avoid error traps. The forensic science community can benefit from the application of lessons learned from human factors work in aviation and medicine to reduce the consequences and likelihood of human error in the scientific interpretation of evidence. While in most instances the results of human error are harmless and correctable, in circumstances such as forensic analysis where errors may lead to the loss of life or liberty, error prevention is imperative.

The National Institute of Justice and the National Institute of Standards and Technology have partnered to sponsor a series of expert working groups to examine the effects of human factors in forensic analyses and recommend approaches to improve practices and reduce the likelihood of errors. This presentation will focus on human factor issues in the handwriting examination process; interpretation and evaluation of handwriting evidence; reporting and testimony; quality assurance and quality control; education, training, and certification; and management. The Expert Working Group on Human Factors in Handwriting Examinations met eight times over the course of two-and-one-half years and heard presentations from experts in the areas of human factors; the weight of evidence in law, statistics, and forensic science; decision making and formulation of propositions; probabilities and likelihood ratios; and other relevant topics. With illustrative examples, this discussion will provide general themes of human factors research, a description of the current handwriting examination process, and present the recommendations of the Working Group.

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**Handwriting Examination, Human Factors, Managing Error**

### **K1 An Improvement for High Sensitivity of Drug Screening by Thermal Desorption and Pyrolysis Combined With Direct Analysis in Real Time-Mass Spectrometry (TDP/DART®-MS)**

Hiroko Abe, MA, University of Chiba, Inohana, 1 8 1, Chuo-ku, Chiba-shi 260-0856, JAPAN; Chikako Takei\*, BioChromato Inc, 1 12 19, Honcho, Fujisawa-shi, Kanagawa-ken 251-0053, JAPAN; Motoshi Sakakura, PhD, AMR, Inc, 2 13 18, Nakane, Meguro-ku 152-0031, JAPAN; Teruhisa Shiota, AMR, Inc, 2 13 18, Nakane, Meguro-ku 152-0031, JAPAN; Kayako Suga, AB Sciex, 4 7 35, Kitashinagawa, Shinagawa-ku 140-0001, JAPAN; Daisuke Yajima, MD, University of Chiba, 1 8 1, Inohana, Chuo-ku, Chiba-shi 260-0856, JAPAN; Hirotaro Iwase, PhD, University of Tokyo, 7 3 1 Hongo, Bunkyo-ku 113-0033, JAPAN

**Learning Overview:** After attending this presentation, attendees will understand the value of TDP/DART®-MS for the rapid identification and screening of forensic drugs in biological and autopsy specimens (e.g., urine and blood).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by explaining how TDP/DART®-MS can be effectively applied as an identification and screening technique for the forensic drugs present in biological and autopsy specimens.

**Introduction:** Drugs present in biological and autopsy specimens cannot be detected without first selecting the pretreatment and analytical conditions appropriate for the drugs. However, in recent years, the situation in which new substances appear one after another, including New Psychoactive Substance (NPS) that threaten society, makes it very difficult to optimize the analytical conditions for each new substance. Thus, there is a need for a comprehensive analysis system that requires minimal investigation of pretreatment and analytical conditions. So, an analytical method for directly analyzing drugs in blood that does not require any pretreatment is being investigated. In a previous study, TDP/DART®-MS was used to separate and detect drugs in urine.<sup>1</sup> The detected ions were correctly identified according to their measured accurate mass and product ion spectra. Moreover, for the quantitative analysis, urine calibrator curves were prepared at concentrations ranging 0.01 µg/ml–1 µg/ml and the curves were linear in that range. However, the detection sensitivity was not satisfactory, so the current investigation aims to improve the detection intensity of drugs.

**Materials and Method:** The samples were standard drug mixture solutions and drug mixture-fortified blood and urine. The standard drug mixture solution consisted of 38 kinds of illegal drugs (e.g., cationics, cannabinoids, and phenethylamines). Mass spectra were obtained by using a quadrupole Time-Of-Flight (qTOF) mass spectrometry equipped with a DART® ion source and a TDP unit. The TDP unit was mounted between the DART® ion source and the mass spectrometry. Mass spectra were measured in positive-ion mode after the samples were heated from room temperature to 300°C. Additionally, to improve the detection intensity, the following two methods were considered: the solvent extraction for deproteinization treatment, and modifications to the analysis systems. In the former case, ethanol (EtOH), methanol (MeOH), and acetonitrile (ACN) were used and in the latter case, the glass tee-tube (the HOOD) was attached between an ion source and the qTOF. This glass tee-tube can work for preventing the diffusion of volatilized drugs from the blood samples.

**Results and Discussion:** Each drug was separated and detected through thermal gradient heating for all samples, and thermal desorption profiles were highly reproducible for individual drugs. The detected ions were correctly identified according to their measured accurate mass and product ion spectra. ACN was the best for deproteinization treat for this investigation, since ACN was attained the highest sensitivity. In addition, when using the HOOD, the peak area of the extracted ion current gram of each drugs was more intense, and it was thought that the volatilized drugs had been ionized more efficiently by attaching the HOOD. Moreover, the calibration curve was linear regardless of the presence or absence of HOOD. Finally, it was confirmed that the heating rate of the TDP device was also effective for improving the detection intensity of drugs, as increasing the heating rate has improved the separation of the peaks and improves the peak intensity.

#### **Reference(s):**

- <sup>1</sup> Hiroko A. et al. Illegal Drugs Analysis by Thermal Desorption and Pyrolysis Combined With Direct Analysis in Real Time-Mass Spectrometry (TDP/DART®-MS). *Proceedings of the American Academy of Forensic Sciences*, 69<sup>th</sup> Annual Scientific Meeting, New Orleans, LA. 2017.

#### **Drug Screening, TDP/DART®-MS, Urine and Blood**



## K2 New $\beta$ 2-Agonists in Sports Doping

Anil Aggrawal, MD\*, Maulana Azad Medical College, New Delhi 110002, INDIA

**Learning Overview:** After attending this presentation, attendees will gain insight into new  $\beta$ 2-agonists that are being used in sports doping. Attendees will also learn about the investigational aspects of these new doping agents.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by stimulating further inquiry and research into the investigational aspects of new  $\beta$ 2-agonists being used in sports.

$\beta$ 2-Agonists are derivatives of phenyl- $\beta$ -ethanol amines with different substituents on the aromatic ring and on the terminal amino group. The exact positions of different substituents further characterize  $\beta$ 2-agonists as catecholamines, resorcinols, or saligenins.

The  $\beta$ 2-agonists exist as two drug classes: the Short-Acting  $\beta$ 2-Agonists (SABA) (first generation, e.g., salbutamol, terbutaline, fenoterol, isoproterenol), and the Long-Acting  $\beta$ 2-Agonists (LABA) (second generation, e.g., salmeterol, formoterol). Recently, the term “Ultra-LABA” has been used to describe the third-generation products of this drug class. In addition to novel Ultra-LABAs, Long-acting Antimuscarinic Agents (LAMAs), phosphodiesterase inhibitors, and Inhaled Corticosteroids (ICSs) are utilized in combination therapy. Using drugs in combination may lower doses of individual agents, decrease adverse effects, simplify medication regimens, and improve compliance.

The most prominent representatives of  $\beta$ 2-agonists are clenbuterol and salbutamol. They are used primarily for the treatment of asthma and related bronchial spasms. Because about 10%–15% of Olympic athletes exhibit asthma syndromes, the use of  $\beta$ 2-agonists is relatively high among them.  $\beta$ 2-agonists exhibit muscle anabolic and lipolytic properties not as their intended effects, but as side effects. Because of relatively low adverse effects of the  $\beta$ 2-agonists, they are considered by some athletes as a “safe” alternative to anabolic androgenic steroids.

Interestingly, although demonstrable in animal experiments, there is little evidence that  $\beta$ 2-agonists can really improve performance in trained athletes. Despite this, in recent years several new  $\beta$ 2-agonist molecules have emerged. Some of these are AZD-3199, AZD-9708, carmoterol, indacaterol, LAS-100977, milveterol, olodaterol, PF-610355, trantinterol, and vilanterol.

In some countries,  $\beta$ 2-agonists are even used to increase the muscle mass of animals, so they can yield more meat. Thus, athletes visiting these countries either for training or simply for tourism may inadvertently consume meat derived from such animals. This may cause them to test positive for  $\beta$ 2-agonists. The German national anti-doping agency has even published a clenbuterol (the most controversial  $\beta$ 2-agonist) warning for athletes traveling to Mexico or China recommending particular vigilance with food.

This presentation discusses the use of  $\beta$ 2-agonists in sports doping, in particular, the new molecules as mentioned above, as well as their use in cattle fattening.

### Doping, Sports, Toxicology

## K3 Screening and Quantitation of Pesticides in Biological Specimens

Muhammad Taimoor Chaudhary, MPhil\*, Punjab Forensic Science Agency, Lahore, Punjab, PAKISTAN; Mohammed Sarwar, PhD, Cuyahoga County Coroner's Office, Cleveland, OH 44106; Sardar Ali Wattoo, MPhil, Punjab Forensic Science Agency, Lahore, Punjab 53700, PAKISTAN; Mohammad A. Tahir, PhD, Punjab Forensic Science Agency, Lahore, Punjab, PAKISTAN

**Learning Overview:** After attending this presentation, attendees will be able to understand: (1) the ongoing potential of Gas Chromatography (GC) coupled with Mass Spectrometry (MS) in the detection of common pesticides, (2) suitable single-step Liquid-Liquid Extraction (LLE) procedures, and (3) the current misuse of different types of pesticides used in Pakistan. The objective of this study was to develop a simultaneous screening and quantitation method for the detection of pesticides by using a suitable solvent combination (for LLE) in the absence of a color test.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the results of the case studies regarding the use of pesticides for suicidal and homicidal purposes. Rapid GC/MS run with Selected Ion Monitoring (SIM) and scan mode for the analysis of common pesticides has not been previously reported and may be highly valued in clinical and forensic settings.

Pesticides are a common source of oral and inhaled poisoning in Pakistan. Cases have been reported regarding accidental and/or intentional poisoning of some commonly used pesticides. Rapid identification of pesticides in biological samples is not only necessary for early treatment decisions but also important for forensic toxicology cases.<sup>1</sup> Analytical methods devised for the determinations of pesticides in food and plant items are not directly useful for biological matrices as sample clean up pours are a major hindrance.<sup>2-10</sup> Despite the benefits of Solid Phase Extraction (SPE), Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS), pressurized liquid extraction and Solid Phase Microextraction (SPME), LLE is still the most commonly used extraction technique for the past two decades.<sup>11</sup>

Basified postmortem samples (2mL) were extracted in a single step by using ethyl acetate and dichloromethane (5mL of 1:1 v/v). Caffeine was used as the internal standard in this study. All extracts were reconstituted with ethyl acetate and hexane in (1:1 v/v), and 1 $\mu$ L was injected in GC/MS. Electron Impact Mass Selective Detector (MSD) was used on Selected Ion Monitoring (SIM) and scan mode whereby DB-5ms capillary column (30m x 250 $\mu$ m x 0.25 $\mu$ m) was installed in GC. The method was validated for screening and quantitation of carbofuran, atrazine, chlorpyrifos, buprofezin, bifenthrin, pyriproxyfen, lambda-cyhalothrin, cypermethrin, deltamethrin, acephate, acetamiprid, and imidacloprid.

The method was validated by using spiked synthetic blood samples with five-point calibration (calibration range 0.50mgL–3.75mg/L). Limit Of Detection (LOD) ranged from 0.202mgL–0.657mg/L, while the Limit Of Quantitation (LOQ) varied from 0.50mgL–1.00mg/L. Accuracy (82.0%–115.20%) and precision (as %CV; 2.14%–17.50%) at three different concentration levels (1.0mgL, 2.5mgL, and 3.75mg/L of pesticides) were within acceptable ranges (80%–120% and < 20%, respectively). Postmortem samples of homicide and suicide cases submitted were analyzed for the presence and quantitation of pesticides. Various cases were found to be positive for the stated pesticides in whole blood, gastric contents, and liver tissues.

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### Pesticides, Homicides, Suicides



### **K4 Elemental Analysis of Hair and Tissue by Open Vessel Digestion and Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) Analysis**

*Beth M. Slaybaugh, MPS\*, NMS Labs, Willow Grove, PA 19090; Donna M. Papsun, MS, NMS Labs, Willow Grove, PA 19030; Riley Murphy, PhD, NMS Labs, Willow Grove, PA 19090; Joan Schemmer, NMS Labs, Willow Grove, PA 19090; Lee M. Blum, PhD, NMS Labs, Willow Grove, PA 19090*

**Learning Overview:** After attending this presentation, attendees will have gained knowledge about a newly developed sample preparation method for the analysis of hair and tissue samples using an open vessel acid digestion method followed by ICP/MS analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a method of acid digestion that provides the opportunity for greater sample throughput and the potential for less contamination compared to closed vessel microwave systems.

Trace metal analysis is highly important in both forensic and clinical toxicology. Metals analysis can play a key role in death and poisoning investigations as well as in environmental and workplace exposure monitoring. Blood, serum, and urine are typical matrices used for analysis. However, in some cases, it is necessary to analyze non-aqueous matrices, such as hair, nails, and tissues. This study introduces a method in which non-aqueous samples are digested into an aqueous solution utilizing a series of chemicals and a heating block, and then analyzed by ICP/MS. Approximately 0.3–1.0 grams of solid sample is weighed into pre-cleaned disposable tubes for the digestion process. Minimization of contamination is key in trace metals analysis for proper identification of toxicological results without the influence of pre-analytical variables and, in this method, digestion tubes are pre-cleaned to remove possible contaminants. These digestion vessels are not recycled as in microwave procedures. An acid digestion mixture composed of 4% hydrochloric, 23% nitric, and 1% hydrofluoric acids is added to each tube, followed by the addition of hydrogen peroxide. The sample is then placed into the heating block that has a 47-sample capacity for digestion, which runs 3 hours and 35 minutes. Upon completion, the sample is diluted to a final volume of 25 milliliters with 2.0% nitric acid. A quantitative method using quadrupole ICP/MS with collision cell technology is used to detect 14 elements. These elements are chromium, manganese, cobalt, nickel, arsenic, selenium, molybdenum, cadmium, tin, antimony, barium, thallium, lead, and bismuth. This presentation will focus solely on barium, arsenic, lead, and cadmium in hair.

Precision and bias estimates were tested for all elements using certified reference materials, including hair, mussel, scallion, celery, dogfish liver, and synthetic urine and blood. Precision as % Coefficient of Variation (CV) for the total digest procedure was less than 15% for all certified reference materials. The accuracy for the total digestion procedure was determined to be within +/-15%. The method uses aqueous standard calibration curves for each analyte of interest. The curves for all elements pass an acceptance criteria in which the slope of the back-calculated concentrations to the target concentrations during validation fell between 0.85 to 1.15 and the bias was within 20%. The relationship between the measured and expected values was also plotted and established to be linear for all elements within the analytical measurement range.

Cases where this method was used include hair analyses over a period of one year from individuals with unknown histories of metals exposures. The findings were: for cadmium ( $n=10$ ), results ranged from 0.018mcg/g to 0.57mcg/g with a median of 0.10mcg/g and average of 0.20mcg/g; for barium ( $n=13$ ), results ranged from 1.6mcg/g to 8.4mcg/g with a median of 4.9mcg/g and average of 4.8mcg/g; for arsenic ( $n=5$ ), results ranged from 0.22mcg/g to 1.2mcg/g with a median of 0.85mcg/g and average of 0.78mcg/g; and for lead ( $n=36$ ), results ranged from 0.14mcg/g to 3.8mcg/g with a median of 0.68mcg/g and average of 0.90mcg/g. Results from hair analysis can help differentiate between baseline levels of trace metals and overexposure or poisoning situations.

In conclusion, open vessel hot block digestion provides a suitable process in which non-aqueous matrices are prepared for ICP/MS analysis. The results from these analyses may assist with the assessment of trace metal determinations in alternative specimens of toxicological interest.

#### **Metals Analysis, Digestion, ICP/MS**





### **K5 The Application of Carboxylic Acid Functionalized Multi-Walled Carbon Nanotubes (COOH-MWCNTs) in the Extraction of Drugs in Plasma**

*Hee-Sun Chung, PhD\**, Graduate School of Analytical Science and Technology, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, SOUTH KOREA; *Heesun Moon, BS*, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon, SOUTH KOREA

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**THIS ABSTRACT WAS NOT PRESENTED.**



### **K6 The Simple and Fast Extraction of Pesticides Using Dried Blood Spot (DBS) and Liquid Chromatography/Quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS)**

*Hee-Sun Chung, PhD\*, Graduate School of Analytical Science and Technology, Chungnam National University, Daejeon 305-764, SOUTH KOREA;  
Sohee Jung, BS, Chungnam National University, Daejeon, SOUTH KOREA*

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**THIS ABSTRACT WAS NOT PRESENTED.**



**K7 The Analysis of Amino Acid in Vitreous Humor Using Liquid Chromatography/Mass Spectrometry (LC/MS) for a Postmortem Interval (PMI) Potential Biomarker**

*Hee-Sun Chung, PhD\*, Graduate School of Analytical Science and Technology, Daejeon, SOUTH KOREA; Sujin Kim, BS, Chungnam National University, Daejeon, SOUTH KOREA*

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**THIS ABSTRACT WAS NOT PRESENTED.**



### **K8 Double-Suicide by Gamma-Butyrolactone (GBL) Ingestion: An Analysis by Headspace/Solid-Phase Microextraction Coupled to Gas Chromatograph/Mass Spectrometer (HS/SPME/GC/MS) and Tandem Columns**

Brian Joseph Waters, MS\*, Fukuoka University, Department of Forensic Medicine, Fukuoka 814-0180, JAPAN; Kenji Hara, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN; Natsuki Ikematsu, BPharm, Fukuoka University, Fukuoka 814-0180, JAPAN; Mio Takayama, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN; Aya Matsusue, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN; Masayuki Kashiwagi, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN; Shin-ich Kubo, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN

**Learning Overview:** After attending this presentation, attendees will better understand the toxicological findings that indicate death due to ingestion of GBL, a metabolic precursor to Gamma-Hydroxybutyrate (GHB).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating an efficient and robust method for the determination of GBL and GHB from biological fluids.

In May of 2018, a man (Case 1) in his early forties went missing from the hospital room where he was staying. He left a handwritten will stating he would die in the backyard of a former male coworker (Case 2) in his late sixties. The note included the name and address of the coworker. Both men were found dead lying side by side on a blue tarp in the backyard of the address written on the will. Near their feet were many empty packages of sleep medication, wine and whisky bottles, unlabeled brown bottles containing an unidentified liquid, and other trash.

Blood and gastric samples from both men were obtained for toxicological analysis. Prior to analysis, ethanol quantitation and screening by Liquid Chromatography/Mass Spectrometry (LC/MS) had been performed by another laboratory. Routine GC/MS screening revealed the presence of GBL, a colorless liquid with a characteristic odor that is often used as a precursor to other chemicals. GBL undergoes rapid enzyme-mediated hydrolysis or oxidation to GHB *in vivo*. The presence of GHB was confirmed by GC/MS analysis after Trimethylsilyl (TMS) derivatization.

For quantitation, duplicate aliquots of 200 $\mu$ L of blood or diluted gastric contents were added to 10-mL headspace vials with an equal volume of distilled water. Sodium chloride was added along with an internal standard, GBL-d<sub>6</sub>; 20 $\mu$ L of 5N hydrochloric acid was added to one sample and 20 $\mu$ L of water was added to the other. The vials were capped and pre-heated for 5min at 70°C. An HS/SPME fiber was used to extract the GBL from the samples. The SPME fiber was inserted and exposed to the headspace and continued to be heated at 70°C for 10min. After adsorption, the fiber was inserted into the GC inlet and desorbed for 2min at 250°C. In the vial containing acid, any GHB in the sample was converted to GBL. In the vial omitting the acid, the GHB was not converted. The difference between the GBL quantitation in the acid sample and the non-acid sample represented the amount of GHB in the specimen.

Separation was performed on a Shimadzu® TQ8030 GC/MS with tandem columns consisting of a Rtx®-200 (5m x 0.15mm x 0.25 $\mu$ m df) pre-column connected serially to an Rtx®-200 (8m x 0.18mm x 0.4 $\mu$ m df) separation column. Injection was in the split mode at 1:15. Helium was the carrier gas with an initial pressure of 259.7kPa. The interface and ion source temps were 260°C and 230°C, respectively. The initial column temp was 60°C for 0.8min, ramped to 250°C at 70°C/min, and held for 3min. The MS was operated in the scan-SIM mode (scan: *m/z* 40-550; SIM: *m/z* 42, 86 for GBL, *m/z* 48, 92 for GBL-d<sub>6</sub>) with electron impact ionization at 70eV.

Case 1 had absolute GBL concentrations in the blood and gastric contents of 75 $\mu$ g/mL and 695 $\mu$ g/mL, respectively. Absolute GHB concentrations (as total GHB-absolute GBL) in the blood and gastric contents of Case 1 were 404 $\mu$ g/mL and 103,955 $\mu$ g/mL, respectively. Absolute GBL concentrations in the blood and gastric contents in Case 2 were 64 $\mu$ g/mL and 782 $\mu$ g/mL, respectively. Case 2 had absolute GHB concentrations in the blood and gastric contents of 367 $\mu$ g/mL and 43,436 $\mu$ g/mL, respectively.

The GBL and GHB levels were consistent with other fatalities attributed to GHB/GBL intoxication in the literature. Both men had several different compounds in their system, including sleep medication and alcohol, in various amounts.

#### **Gamma-Butyrolactone, Gamma-Hydroxybutyrate, Postmortem**



## K9 Validation of the Neogen® Enzyme-Linked Immuno-Sorbent Assay (ELISA) Cocaine/Benzoyllecgonine-2 Kit for Whole Blood and Urine Specimens

Nicholas B. Tiscione, MS\*, West Palm Beach, FL 33406

**Learning Overview:** After attending this presentation, attendees will understand the performance of the Neogen® ELISA Cocaine/Benzoyllecgonine-2 kit for screening whole blood and urine specimens as evaluated by a validation based on the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in forensic toxicology laboratories.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the validation of an ELISA method based on the SWGTOX standard.

**Objective:** The validation of a semi-quantitative method for the rapid screening of whole blood and urine specimens by a Dynex DSX® Automated ELISA System using the Neogen® Cocaine/Benzoyllecgonine-2 kit.

**Method:** Whole blood samples were diluted 1:5 with manufacturer-provided buffer before being loaded onto the instrument, then further diluted 1:2 by the instrument. Urine samples were diluted 1:20 with buffer by the instrument. The diluted sample volume added to each well was 15µL and 10µL for whole blood and urine, respectively. For all other parameters (e.g., incubation times, reagent volumes), the manufacturer assay instructions were followed. Performance of the assay was evaluated at decision points of 50ng/mL and 25ng/mL for whole blood and 300ng/mL, 150ng/mL, and 50ng/mL for urine. The validation included the evaluation of sensitivity, precision, specificity, carryover, plate drift, ruggedness/robustness, and a case sample comparison.

**Results:** Carryover was evaluated by running three replicates of a blank matrix control following a positive matrix control at 10µg/mL for both blood and urine. Carryover was not detected in the assay. The sensitivity was evaluated by replicate analysis of a blank matrix control to determine the theoretical Limit Of Detection (LOD) and by the analysis of standards at successively lower levels to determine an experimental LOD. The theoretical LOD was determined to be 16ng/mL for blood and 23 ng/mL for urine. The experimental LOD was determined to be 25ng/mL for both blood and urine. Precision was evaluated at 0ng/mL, 12.5ng/mL, 25ng/mL, 37.5ng/mL, 50ng/mL, 75ng/mL, and 100ng/mL for blood and 0ng/mL, 25ng/mL, 50ng/mL, 75ng/mL, 150ng/mL, 225ng/mL, 300ng/mL, and 450ng/mL for urine with three replicates at each level over five separate runs ( $n=15$ ). The Coefficient of Variation (CV) was less than or equal to 6.1% for blood and urine at all studied concentrations. The mean response  $\pm 2$  Standard Deviations (SD) at each decision point for both blood and urine at 50ng/mL and 300ng/mL, respectively, did not overlap with the mean response  $\pm 2$  SD of standards prepared at  $\pm 50\%$  of the concentration of the decision points. Overlap was observed at decision points of 25ng/mL in blood and 150ng/mL and 50ng/mL in urine. Specificity was evaluated for cocaine and cocaethylene in blood by the analysis of negative matrix samples fortified at 10µg/mL, 100ng/mL, 70ng/mL, and 50ng/mL. In urine, concentrations of 10µg/mL, 600ng/mL, 400ng/mL, and 300ng/mL were employed. Observed cross reactivity was lower than that reported by the manufacturer. Cocaine cross reactivity was estimated to be 44% for whole blood and 40% for urine. Cocaethylene cross reactivity was estimated to be 46% for whole blood and 43% for urine. No interference was observed from screening known authentic samples, which contained 22 commonly identified drugs and metabolites. Two out of five positive blood specimens and one out of five urine specimens were identified as being negative by the assay, with responses just above the cutoff. These specimens had benzoyllecgonine concentrations near the cutoff and had been in storage for 1–35 months. Plate drift was evaluated by analyzing 24 replicates at the validated decision point for each matrix. The number of replicates analyzed was greater than the number of samples run in routine casework. Plate drift was not observed.

Whole Blood ( $n=15$ )	Level (ng/mL)	Mean O.D.	SD	CV (%)	Mean + 2 SD	Mean – 2 SD
	0	0.898	0.038	4.2	0.974	0.822
	25	0.693	0.031	4.5	0.755	0.631
	50	0.568	0.026	4.6	0.620	0.516
	75	0.460	0.028	6.1	0.516	0.404
	100	0.408	0.023	5.6	0.454	0.362

Urine ( $n=15$ )	Level (ng/mL)	Mean O.D.	SD	CV	Mean + 2 SD	Mean – 2 SD
	0	1.166	0.044	3.8	1.254	1.078
	150	0.579	0.030	5.2	0.639	0.519
	300	0.407	0.017	4.2	0.441	0.373
	450	0.310	0.019	6.1	0.348	0.272

**Conclusion:** The Neogen® Cocaine/Benzoyllecgonine-2 ELISA kit is a precise, specific, and rapid screening procedure to detect benzoyllecgonine in blood and urine.

### Cocaine, Validation, Enzyme-Linked Immuno-Sorbent Assay



### K10 The Detection of Various Drugs in Human Urine Samples Via Total Vaporization-Solid Phase Microextraction (TV-SPME)

*Kymeri E. Davis, BSc\*, Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202; John V. Goodpaster, PhD, FIS Program, Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202*

**Learning Overview:** After attending this presentation, attendees will understand: (1) TV-SPME and how it may be used to detect drug analytes, and (2) the ability of Gas Chromatography/Mass Spectrometry (GC/MS) and TV-SPME to detect drugs in toxicological samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how TV-SPME may be implemented in laboratories.

While GC/MS is a frequently used technique in forensic science laboratories, there are limitations, such as the need for compounds to be thermally stable as well as volatile. Some compounds must undergo derivatization prior to being injected into the gas chromatogram to satisfy these requirements. SPME is a technique in which analytes are absorbed onto a fiber that is then placed inside the GC inlet for desorption. TV-SPME utilizes the same technique as standard SPME but vaporizes a sample extract that is then absorbed onto the fiber. The Polydimethylsiloxane-Divinylbenzene (PDMS-DVB) fiber is first exposed to a vial containing the derivatization agent. The SPME fiber is then exposed to a new vial containing the analyte of interest. A study was conducted to determine the optimal time needed for the fiber to absorb the derivatization agent so that both the solvent (e.g., water) and the analyte were fully derivatized with no underivatized compounds detected. This study indicated that 50 minutes was the optimal time needed to adequately saturate the fiber. TV-SPME is beneficial because it allows for the analyte to be derivatized during the extraction process, which reduces analysis time. TV-SPME requires little sample preparation and small sample sizes.

It was hypothesized that TV-SPME could be used to analyze drugs and drug metabolites in human urine samples. The GC temperature program was set to an initial temperature of 60°C with a 15°C/min oven ramp. The inlet temperature was 250°C using splitless mode. The MS scan range was 40m/z–550m/z. Drugs of interest included methamphetamine and amphetamine. Each drug was spiked into a human urine sample known to be free of any illegal substances. Concentrations were at ng/mL levels. Each sample was then analyzed using a TV-SPME GC/MS method after being exposed to the proper derivatization agent for a sufficient amount of time. After exposing the SPME fiber to Trifluoroacetic Anhydride (TFAA) for 50 minutes, methamphetamine (28.6ng/mL) and amphetamine (19.0ng/mL) derivatives were both successfully detected in a urine sample.

Currently, this method shows that methamphetamine and amphetamine can be detected at ng/mL levels in human urine with no prior sample preparation aside from placing the sample into a headspace vial. Future work will include a recovery analysis and future compounds may include benzoylecgonine,  $\gamma$ -Hydroxybutyric Acid (GHB),  $\gamma$ -Butyrolactone (GBL), and 11-Nor-9-Carboxy- $\Delta^9$ -Tetrahydrocannabinol (THC-COOH). There are currently numerous methods and techniques available for the analysis of controlled substances. However, these methods often require sample preparation such as an extraction. This TV-SPME method requires little-to-no sample preparation and utilizes a simple GC/MS method.

#### Gas Chromatography, Solid-Phase Microextraction (SPME), Drugs

## K11 The Postmortem Distribution of 3,4-Methylenedioxyamphetamine (MDMA) and 3,4-Methylenedioxyamphetamine (MDA) From an Accidental Death Due to MDMA Intoxication

Danylle Kightlinger\*, Office of the Chief Medical Examiner, Washington, DC 20024; Victor W. Weedn, MD, JD, The George Washington University, Washington, DC 20007; Lucas W. Zarwell, MFS, Washington, DC 20011

**Learning Overview:** After attending this presentation, attendees will understand the postmortem distribution of MDMA and MDA from an accidental death due to MDMA intoxication.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an evaluation of the postmortem distribution of MDMA and MDA, as well as the ratio of MDA to MDMA, in various matrices, which could aid in the approach and analysis of other MDMA intoxication cases.

A 29-year-old female was found kneeling, facedown on her bedroom floor approximately two hours after her last known communication and was pronounced dead on scene roughly 40 minutes later. The scene was unremarkable. Approximately 18 hours after the decedent was pronounced dead, a medicolegal autopsy was performed, and specimens were submitted for toxicological analysis.

An expanded panel was conducted on the priority sample (Femoral Blood 1), and electrolyte's testing was conducted by biosensor analysis on vitreous humor. After completion of the expanded panel, all other specimens were analyzed using a confirmatory, quantitative Gas Chromatography/Mass Spectrometry (GC/MS) method for amines.

Medicolegal autopsy findings included: pulmonary edema (1,300 grams combined lung weight); zonal necrosis of the liver; approximately 25mL of green material in the stomach with gastric mucosal hemorrhage; and a contusion on the inner lower lip (a possible indication of agonal seizure activity). Electrolytes results were determined, and from the expanded panel, seven compounds were reported in Femoral Blood 1: MDMA, MDA, cocaine, benzoylecgonine, diphenhydramine, alprazolam, and naloxone. Furthermore, MDMA and MDA postmortem concentrations and ratios for all submitted specimens were as follows:

**Table 1:** MDMA and MDA Postmortem Concentrations and Ratios by Specimen

Specimen	MDMA (mg/L or mg/kg) <sup>^</sup>	or	MDA (mg/L or mg/kg) <sup>^</sup>	or	MDA:MDMA	Fluid or Tissue MDMA: Blood 1 MDMA	Fluid or Tissue MDA: Femoral Blood 1 MDA
Femoral Blood 1	16.3		0.31		0.01	1.00	1.00
Femoral Blood 2	15.1		0.26		0.01	0.92	0.83
Heart Blood 1	18.0		0.33		0.01	1.10	1.06
Heart Blood 2	18.1		0.28		0.01	1.11	0.90
Urine	5.89		ND*		--	0.36	--
Vitreous Humor	10.3		0.12		0.01	0.63	0.38
Liver	45.9		1.05		0.02	2.81	3.38
Brain	39.7		0.74		0.01	2.43	2.38
Gastric Content	774		ND*		--	47.4	--

<sup>^</sup>Fluids were reported in mg/L; tissues were reported in mg/kg.

\*ND – Not Determined

The official cause and manner of death were ruled as an accident due to MDMA intoxication, although it was recognized that suicide was a distinct possibility. Other drugs detected were not significant in quantity and all electrolyte levels were within normal postmortem limits. The extremely high level of MDMA present in the gastric content suggests the route of administration was ingestion. However, no tablets were found in the stomach and the formulation in which the MDMA was ingested—suspected tablet or liquid form—is unknown. MDMA and its active metabolite appear to exhibit similar pharmacokinetics, showing a seemingly consistent ratio with respect to the MDA/MDMA concentrations (0.01–0.02) in all specimens. The liver and brain concentrations were substantially higher than the femoral blood. The low MDMA concentration present in the urine suggests death occurred soon after ingestion. The MDMA postmortem concentrations in this case are some of the highest reported. While femoral blood remains the preferred sample, this data suggests that heart blood concentrations are comparable to femoral blood, indicating heart blood may be used when necessary.

### MDMA, MDA, Postmortem Distribution



### K12 The Detection and Quantitation of Ten Synthetic Cannabinoid Metabolites in Human Urine Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MS/MS)

Cassandra A. Swart, BS\*, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Daniel Lee, MS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Mikayla Caldwell, BS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Katherine N. Moore, MS, Research Triangle Park, NC 27709; Nichole D. Bynum, MS, RTI International, Johnson Building, Research Triangle Park, NC 27709; Sabra R. Botch-Jones, MS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118

**Learning Overview:** After attending this presentation, attendees will be able to use the method described, or develop their own singular method, for analysis of urine for selected synthetic cannabinoid metabolites.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the analysis of selected synthetic cannabinoid metabolites.

**Background/Introduction:** Synthetic cannabinoids remain in the top 25 drug-encountered analytes in seized drug evidence based on the United States Drug Enforcement Administration's National Forensic Laboratory Information System 2017 mid-year report. Despite efforts to control synthetic cannabinoids, illicit manufacturers continue to modify their structures to avoid legal regulations, creating an ever-changing analytical target for forensic laboratories. In addition, due to structural modifications of these synthetic cannabinoids, many of these compounds can bind to endogenous CB1 and CB2 receptors with greater affinity, causing severe adverse and life-threatening effects. Because of their structural dissimilarity to  $\Delta^9$ -Tetrahydrocannabinol (THC), combating the rapid growth and emergence of synthetic cannabinoids with conventional THC-based methods is often not effective.

**Objective:** With a focus on synthetic cannabinoids of different core structures such as naphthoylindole, adamantoylindole, quinoliny, and carboxamide, the purpose of this research was to develop and validate a robust and reliable method to accurately identify and quantify ten synthetic cannabinoid metabolites in human urine.

**Method:** Using HPLC with a 4000 QTRAP® Electrospray Ionization Tandem Mass Spectrometry (ESI/MS/MS) in positive ionization mode, samples were extracted using supported liquid extraction using ISOLUTE® cartridges. The method was validated in accordance to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for quantitative analysis using the following analytes: UR-144 degradant N-pentanoic acid, UR-144 N-(5-hydroxypentyl), PB-22 N-(5-hydroxypentyl), MDMB-FUBICA metabolite 3, JWH 250 N-pentanoic acid, ADB-PINACA pentanoic acid, ADB-PINACA N-(4-hydroxypentyl), AB-FUBINACA metabolite 3, 5-fluoro PB-22 3-carboxyindole, and 5-fluoro MDMB-PICA metabolite 7.

**Results:** With this developed method, total analysis time was eight minutes with samples eluting within 3.26 to 4.47 minutes. Calibration curves for each analyte had accepted  $R^2$  values  $> 0.99$ . The calibration model was established to be linear using a weighting factor of  $1/x$ . A linear dynamic range of 5ng/mL – 40ng/mL was used for all analytes. Extraction of analytes using Supported Liquid Extraction (SLE) cartridge improved sample-prep time by more than half, compared to traditional solid phase extraction methods. Percent recovery of analytes using the SLE was determined to be from 68.4% to 90.7%. Bias and precision was assessed at 5ng/mL, 25ng/mL, and 30ng/mL for all analytes. Samples had acceptable calculated percent bias and percent coefficient of variation within  $\pm 20\%$ . No carryover was observed. No interference was observed for other commonly encountered drugs clonazepam, diazepam, (+) methadone, morphine, fentanyl, cocaine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 25I-NBOMe, and Phencyclidine (PCP) at 2,000ng/mL.

**Conclusion/Discussion:** The overall development and validation of this method demonstrates a sensitive and reliable way to positively identify ten different synthetic cannabinoid metabolites in human urine in rapid time.

#### Synthetic Cannabinoids, Supported Liquid Extraction, Metabolites



### K13 The Detection and Quantitation of 17 Synthetic Cannabinoids in Human Whole-Blood Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Following Supported Liquid Extraction

*Daniel Lee, MS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Shawn Foley, BS, Boston University, Biology Department, Boston, MA 02118; Erika Phung, BS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Cassandra A. Swart, BS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Nichole D. Bynum, MS, RTI International, Research Triangle Park, NC 27709; Katherine N. Moore, MS, Research Triangle Park, NC 27709; Sabra R. Botch-Jones, MS\*, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118*

**Learning Overview:** After attending the presentation, attendees will have information on the validation of a detection and quantitation method of select synthetic cannabinoids in human whole-blood with the use of LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information on the analysis of select naphthoylindole, adamantoylindole, quinolinyl, and carboxamide synthetic cannabinoids in human whole-blood by LC/MS/MS.

**Background/Introduction:** Synthetic cannabinoids have become a growing concern in society. The extensive list of synthetic cannabinoids and the abuse rate has drawn the attention of government agencies throughout the world. These synthetic cannabinoids can adopt several different structures, while still acting on endogenous cannabinoid (CB1 and CB2) receptors. In addition, due to structural modifications of these synthetic cannabinoids, many of these compounds can bind to CB1 and CB2 receptors with greater affinity, causing severe adverse and life-threatening effects. Because of their structural dissimilarity to the phytocannabinoid  $\Delta^9$ -THC, combating the rapid growth and emergence of synthetic cannabinoids with conventional THC-based methods has become an ongoing struggle.

**Objective:** The purpose of this research was to develop and validate a robust and reliable method to accurately identify and quantify 17 synthetic cannabinoids in human whole-blood using LC/MS/MS. The method was validated in accordance to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for quantitative analysis using the following analytes: 4-cyano CUMYL-BUTINACA, 5-fluoro-3,5-ABPFUPPYCA, 5-fluoro ADB-PINACA, 5-fluoro PY-PINACA, ADB-PINACA, APP-PICA, CUMYL-THPINACA, EMB-FUNICACA, JWH-250, MDMB-FUBICA, MEP-CHMICA, MO-CHMINACA, NM2201, PB-22, RCS-8, UR144, and XLR11.

**Method:** Using High Performance Liquid Chromatograph (HPLC) with a 4000 QTRAP® Electrospray Ionization Tandem Mass Spectrometry (ESI/MS/MS) in positive ionization mode, the total analysis time was 8.013 minutes with samples eluting within 3.8 to 5.8 minutes. Calibration curves for each analyte had accepted  $R^2$  values  $> 0.99$  using a weighting factor of  $1/x$ . A linear dynamic range of 0.5ng/mL – 25ng/mL was used for all analytes, except for MO-CHMINACA and NM2201, which were quantifiable at lower (0.1ng/mL) levels. Extraction of analytes was performed using ISOLUTE® Supported Liquid Extraction (SLE) cartridges, which improved sample-prep time by more than half, compared to traditional methods.

**Results:** Percent recovery of analytes using the SLE was determined to be from 54.92% to 83.36%. Bias and precision was assessed at 1ng/mL, 3ng/mL, 7ng/mL, and 20ng/mL for all analytes. All samples had accepted calculated percent bias and percent coefficient of variation within  $\pm 20\%$ . No signs of carry-over were observed with this method. Ionization suppression and enhancement was observed at various levels, from -4.47% to 76.67%, but had little effect on other validation parameters. Analysis of other commonly encountered drugs (clonazepam, diazepam, (+) methadone, morphine, fentanyl, cocaine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 25I-NBOMe, and Phencyclidine (PCP)) at 2,000ng/mL showed false identification for ADB-PINACA.

**Conclusion/Discussion:** The overall development and validation of this method demonstrates a sensitive and reliable way to positively identify 17 different synthetic cannabinoids in human whole-blood in rapid time.

#### Synthetic Cannabinoids, Supported Liquid Extraction, Forensic Toxicology



### **K14 An Analysis of Carboxyhemoglobin in Postmortem Blood and Epidemiology Data of Suicide Autopsy Cases From 2011–2016 in Taiwan**

*Chu-An Yang, MS\*, Institute of Forensic Medicine, New Taipei City 23548, TAIWAN, REPUBLIC OF CHINA; Hsiu-Chuan Liu, MS, Institute of Forensic Medicine, Taipei, TAIWAN, REPUBLIC OF CHINA; Ray H. Liu, PhD, Forensic Science Review, Vancouver, WA 98685; Dong-Liang Lin, PhD, Institute of Forensic Medicine, New Taipei City 23548, TAIWAN, REPUBLIC OF CHINA*

**Learning Overview:** After attending this presentation, attendees will gain insights regarding: (1) factors that are important to the analysis of Carboxyhemoglobin (COHb) in postmortem blood specimens, and (2) trends and epidemiological data of Carbon Monoxide (CO) poisoning deaths in Taiwan during the 2011–2016 period.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving forensic laboratories' analysis of COHb in postmortem blood specimens and helping medical examiners' determination on cause of death in CO poisoning cases.

Poisoning-related death statistics in Taiwan, regularly provided by the Ministry of Justice's Institute of Forensic Medicine, indicated the number of CO-poisoning cases has been consistent and significant in recent years. The most common cases were suicidal deaths facilitated by coal burning. The measurements of COHb in postmortem blood specimens could be unreliable and difficult due to putrefaction and other factors. The purpose of this study was to evaluate whether test results could be affected by: (1) blood from different sources (heart, pelvis, and thoracic cavity); and (2) specimen storage temperature (–20°C, 4°C, 25°C, and 40°C). In addition, suicide cases (with autopsy) were studied to reveal the trend of CO-poisoning deaths in Taiwan during the 2011–2016, six-year period to aid in the formulation and administration of suicide prevention policies in Taiwan.

The ABL80 FLEX CO-OX blood gas analyzer was used to determine COHb concentration in test specimens, following the operation guide provided by the manufacturer. It was found to be easy to operate, requiring a short test time and small sample volume (around 70µL). Test results indicated: (1) the ranges of intra-/inter-day precision and accuracy were 0.00%–6.37% and 98.1%–104.7%, respectively; (2) the best results for postmortem blood specimens came with two-fold dilution (with deionized water and 0.9% sodium chloride aqueous solution), as specimens provided were often too thick with a small volume; (3) no significant difference was found among specimens collected from different parts of the same person; and (4) COHb concentration was stable through 28 days when specimens were refrigerated at –20°C or 4°C.

This method was successfully applied to the analysis of postmortem blood specimens from forensic cases in Taiwan for the 2011–2016 period. Statistics of autopsy cases (404 total) during this six-year period revealed: (1) the number of suicidal deaths beginning with 2011 were: 17, 19, 18, 30, 18, and 18 (120 total); (2) there were 108 CO-poisoning suicides by coal burning—nearly 90% of the total number of suicide cases; (3) the number of male victims was significantly higher than female (272 vs. 132); (4) most victims (71) were 31–40 years old; (5) the peak periods were January/February and September/October; and (6) most cases were found in residence. The range, mean, and median of COHb concentrations found in the 120 suicide case specimens were 10.1%–91.1%, 59.9%, and 62.9%, respectively. Alcohol, sedatives, and antidepressants were the most commonly found drugs in these specimens, but with concentrations below their respective lethal levels.

#### **Carboxyhemoglobin (COHb), Postmortem Blood Specimen, Epidemiology**



### K15 An Imidacloprid Insecticide Fatal Poisoning: Gas Chromatography/Mass Spectrometry (GC/MS) Detection in Alternative Biological Matrices

*Antonella Sorrentino, MD, Istituto Medicina Legale, Bari 70124, ITALY; Biagio Solarino, PhD, Università degli Studi di Bari, Bari 70125, ITALY; Silvia Trotta\*, Institute of Legal Medicine, Bari 70124, ITALY; Quintino Mita, BS, Laboratory of Forensic Toxicology, Bari 70124, ITALY; Giuseppe Strisciullo, BES, University of Bari, Bari 70124, ITALY; Lucia Aventaggiato, Policlinico, Bari 70100, ITALY*

**Learning Overview:** After attending this presentation, attendees will better understand the chloronicotinoid compounds, including its intended uses, toxic effects, and, because of its availability in certain communities, its use as a suicide modality.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by stressing the importance of scene investigation, autopsy findings, and toxicology results to determine the cause of death in imidacloprid (pesticide) intoxication.

Imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine) is a neonicotinoid insecticide in the chloronicotinyl nitroguanidine chemical family, rapidly and almost completely absorbed after ingestion. It is subsequently metabolized to 6-chloronicotinic acid, which is conjugated with glycine, then eliminated or reduced to guanidine. About 70% to 80% of an administered dose is excreted unchanged in the urine and 20% to 30% in feces. Imidacloprid acts on the Central Nervous System (CNS) as an agonist at the nicotinic acetylcholine receptor. It causes initial stimulation followed by fatigue of the agonized neurons and ultimately interferes with the transmission of neuronal impulses. Insecticide products containing imidacloprid are used worldwide; therefore, occasionally, accidental intoxication or intentional self-intoxication occurs throughout the world. Despite the original belief that imidacloprid has low mammalian toxicity, there is increasing evidence that imidacloprid may cause heart, kidney, and other organ damages and even death in addition to gastrointestinal irritation and neurological symptoms.

**Case:** A 55-year-old man was found dead lying prone on the grass near his summerhouse. No signs of traumatic injury were on the body surface and the parents denied serious systemic disorders. A plastic bottle of insecticide containing a little quantity of thick, white liquid was in the kitchen, while close to the body there were traces of this substance, apparently vomited by the victim. A medicolegal autopsy was required. The man's length was 171cm and he weighed 79kg. There were no signs of traumatic injury on the body. His face showed congestion, and there was some viscous, white fluid in his mouth. A fluid material with the same organoleptic features was at a section in the upper airways, esophagus, in the stomach (ca. 300cc), and in the duodenum. No other macroscopic alteration of the inner organs was observed. Microscopic examinations revealed pulmonary edema, fragmentation of myocardial fibers, and slight pancreatic hemorrhage. Full scan GC/MS analyses were performed on an Agilent® 6890 GC coupled 5973 inert mass spectrometer, previous liquid-liquid extraction of the biological specimens by organic mixture solvents (n-heptane; 1,2-dichloroethane; dichloromethane; 2-propanol) at pH 1.5. Both imidacloprid (IMI) and its metabolite 6-chloronicotinic acid (6-CNA) were quantified: the retention time was respectively 11, 14, and 5.64 minutes, while the ions spectrum was 211, 126, 99 (IMI) and 214, 170, 140 (6-CNA). For the detection of the metabolite, the derivation procedure BSTFA-1% TCMS was needed. Analytical results showed: inferior vena cava blood (IMI: 35µg/ml; 6-CNA: 0.6µg/ml), femoral blood (IMI: 55µg/ml; 6-CNA: 0.76µg/ml), urine (IMI: 14µg/ml; 6-CNA: 0.79µg/ml), vitreous humor (IMI: 18µg/ml; 6-CNA: 0.11µg/ml), abdominal adipose tissue (IMI: 28µg/g; 6-CNA: 0.39µg/g), and Total Gastric contents (IMI: 105mg; 6-CNA: 5.1mg).

**Discussion:** Acute toxicity is generally measured by LD50 and LC50 but these values are unknown for imidacloprid. Hence the presence of this substance in the plastic bottle and its distribution in all the alternative biological matrices here analyzed suggest an uncommon fatal intoxication of imidacloprid. Regarding the manner of death, no farewell letter was near the cadaver and the parents denied any kind of trouble so difficult as to explain suicide. However, the thorough scene investigation coupled with external examination and autopsy are in accordance with an intentional ingestion of pesticide.

#### Imidacloprid, Alternative Biological Matrices, Suicide



### K16 An Impaired Driving Case Involving Xylazine

*Ilene K. Alford, MS\*, Palm Beach County, West Palm Beach, FL 33406; Nicholas B. Tiscione, MS, West Palm Beach, FL 33406*

**Learning Overview:** After attending this presentation, attendees will be aware of the appearance of xylazine in combination with fentanyl, cocaine, and other drugs in a specimen collected as part of an impaired driving investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into the rapidly evolving trends in drug use and abuse.

**Objective:** To describe an impaired driving case involving xylazine identified in a urine specimen in combination with fentanyl, cocaine, and other drugs.

**Background:** Xylazine is an alpha2 adrenergic agonist used in veterinary medicine for sedation, anesthesia, muscle relaxation, and analgesia. Recreational use of xylazine has been reported in Connecticut, Puerto Rico, Texas, and in Europe. Xylazine has been previously reported in cases involving heroin and fentanyl in Philadelphia, PA. Recently, xylazine in combination with fentanyl has also been identified in Palm Beach County, FL, and Ohio in drug seizure cases.

**Method:** A urine specimen was collected and submitted as part of an impaired driving investigation after a Breath Alcohol Analysis (BrAC). The urine specimen was screened using a basic extraction with scan Gas Chromatography/Mass Spectrometry (GC/MS) and a nine-panel Enzyme-Linked Immunosorbent Assay (ELISA) for barbiturates, benzodiazepines, buprenorphine, carisoprodol, cocaine/benzoylecgonine, fentanyl, opiates, oxycodone/oxymorphone, and cannabinoids. All positive results were confirmed with GC/MS and/or Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

**Results:** The incident occurred at approximately 5:00 p.m. on a Saturday evening. The subject was a 29-year-old White male. Witnesses observed him running in front of cars in a parking lot and beating on vehicles. An officer in a marked patrol vehicle arrived on the scene and observed the subject behind the wheel making a couple of failed attempts to back into parking spaces. The officer made successive efforts to stop the suspect's vehicle by activating her emergency lights, air horn, and Public Address (PA) system giving verbal instructions to stop the vehicle, all of which were initially unsuccessful. The suspect finally stopped after the officer's second use of the air horn. When the officer approached the subject's vehicle on foot, the subject was sweating profusely, had red, glassy eyes, miosis, stuttering and excited speech, and could not stop fidgeting and flailing his arms. The subject had difficulty turning off the vehicle when requested, attempting to do so with the gear shifter and wipers switch before the officer advised him to use the ignition switch. Upon exiting the vehicle, the subject was unsteady on his feet. Field sobriety exercises were administered. During the horizontal gaze nystagmus task, the subject closed his eyes several times leaving them closed for several seconds while his head fell backward. Lack of smooth pursuit was observed in both eyes. During the other tasks, the subject had difficulty maintaining his balance and completing the tasks as instructed. A brown powder that field tested positive for heroin and a syringe was in the vehicle.

The BrAC did not detect ethanol. Cocaine, fentanyl, codeine, morphine, buprenorphine, xylazine, delta-9-carboxy-tetrahydrocannabinol, lidocaine, and tramadol were identified in the urine specimen. Benzoylecgonine was indicated but not confirmed due to the presence of cocaine.

**Conclusion:** Recent submissions to the Palm Beach County Sheriff's Office Drug Chemistry Unit involving xylazine have included a counterfeit M30 oxycodone tablet containing methamphetamine, fentanyl and xylazine, and a tan powder containing heroin, fentanyl, and xylazine. Xylazine was tentatively identified by library match in both cases. This is consistent with previously reported cases involving concurrent detection of xylazine with heroin and fentanyl and indicates xylazine may be an additive in samples involving heroin and opioids.

#### Xylazine, Impaired Driving, Case Report



## K17 Liquid Chromatography/Quadrupole Time-Of-Flight (LC-QTOF) Screening for Fentanyl Analogs in Whole Blood and Oral Fluid

Kaitlyn B. Palmquist, BS\*, Huntsville, TX 77340; Madeleine J. Swortwood, PhD, Sam Houston State University, Huntsville, TX 77341

**Learning Overview:** After attending this presentation, attendees will better understand the role of high-resolution Mass Spectrometry (MS) in the identification of novel fentanyl analogs in biological samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a unique screening methodology for the detection of fentanyl analogs in blood and oral fluid.

Fentanyl analogs are responsible for an increasing number of opioid related deaths in the United States. Routine forensic analyses are often unable to detect these analogs due to the low concentrations and similar molecular structures. To address this problem, a comprehensive screening method was developed and validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for 14 fentanyl analogs in whole blood and oral fluid using LC-qTOF/MS.

Blood (0.25mL) or oral fluid (1mL containing 1:3 oral fluid: Quantisal® buffer) were fortified with deuterated internal standards, diluted with phosphate buffer, and extracted with a polymeric solid-phase extraction column. After washing, analytes were eluted with 5% ammonium hydroxide in ethyl acetate, dried, and reconstituted in mobile phase. An Agilent® Technologies 1290 Infinity® LC coupled to an Agilent® Technologies 6530 Accurate Mass TOF/MS was used for analysis in two separate acquisition modes: TOF mode and All Ions Fragmentation (AIF) mode. Personal Compound and Database Libraries (PCDLs) were produced in-house containing all analytes of interest ( $n=14$ ), as well as other drugs of abuse ( $n=53$ ). Both matrices were validated according to SWGTOX Standard Practices for Method Validation in Forensic Toxicology guidelines. For proof of applicability, authentic postmortem blood ( $n=30$ ) and antemortem oral fluid samples ( $n=18$ ) were extracted and analyzed as described above.

Chromatographically, all analytes eluted before seven minutes. Baseline resolution was achieved for most analytes. Butyrylfentanyl and isobutyryl fentanyl were unable to be distinguished by the LC system, which is a common issue encountered in toxicological analyses. To address this problem, certified reference standards were analyzed using an Agilent® Technologies 6890N Gas Chromatograph (GC) coupled to an Agilent® Technologies 5975B MS equipped with an Agilent Technologies DB-5 (30m x 0.25mm x 0.25 $\mu$ m) column. The GC was able to fully separate these isomeric compounds 12.5min method.

The Limits Of Detection (LOD) for all analytes in blood were 0.1-0.25ng/mL and 0.1-1.0ng/mL in TOF and AIF modes, respectively. In oral fluid, the LOD were 0.25ng/mL and 2.5ng/mL in TOF and AIF modes, respectively. No carryover or interferences (exogenous or endogenous) were observed. Matrix effects in blood were considered acceptable for most analytes with minor ion enhancement of 1%–14.4%, while the nor- analytes (metabolites) exhibited ion suppression >25%. Matrix effects in oral fluid were considered acceptable for all analytes with ion suppression and enhancement ranging from -11.7%–13.3%. Stability was assessed after 24 hours in the autosampler (blood at 22°C and oral fluid at 4°C) and refrigerator (blood at 4°C). All analytes were determined to be stable under each condition, except alfentanil in OF (>25% loss in autosampler). Authentic postmortem blood samples ( $n=30$ ) were positive for: furanyl fentanyl ( $n=16$ ), 4-ANPP ( $n=15$ ), cis-methyl fentanyl ( $n=4$ ), fentanyl ( $n=3$ ), and valeryl fentanyl ( $n=1$ ). Additional drugs of abuse detected included methamphetamine ( $n=2$ ), cocaine ( $n=2$ ), ketamine ( $n=2$ ), 6-MAM ( $n=7$ ), alprazolam ( $n=5$ ), morphine ( $n=8$ ), codeine ( $n=7$ ), hydrocodone ( $n=2$ ), etizolam ( $n=3$ ), amitriptyline ( $n=1$ ), buprenorphine ( $n=1$ ), zolpidem ( $n=1$ ), meperidine ( $n=1$ ), and U-47700 and its metabolites (n-desmethyl-U47700 and n,n-didesmethyl-U47700) ( $n=15$ ). Oral fluid samples ( $n=18$ ) collected from arrestees under an Institutional Review Board (IRB) -approved protocol did not contain any fentanyl analogs. However, additional drugs of abuse detected included methamphetamine ( $n=15$ ), amphetamine ( $n=7$ ), cocaine ( $n=4$ ), 6-MAM ( $n=4$ ), morphine ( $n=2$ ), codeine ( $n=1$ ), alprazolam ( $n=1$ ), and mephedrone ( $n=1$ ).

This research presents two validated screening methods for fentanyl analogs in whole blood and oral fluid using LC-qTOF analysis with low limits of detection. In addition, this research presents an alternative GC/MS application for the separation of fentanyl analogs, specifically, butyryl and isobutyryl fentanyl.

This research was funded by the Forensic Science Foundation Lucas Grant Award and a grant from the National Institute of Justice (NIJ).

### Fentanyl Analogs, Postmortem Blood, Oral Fluid



### K18 Kava Impairment in Drivers

Ashley A. Gilbert, BS\*, Sam Houston State University, Huntsville, TX 77340; Justin Grodnitzky, PhD, Iowa Division of Criminal Investigation Criminalistics Laboratory, Ankeny, IA 50023; Jonna Berry, PhD, Iowa Division of Criminal Investigation Criminalistics Laboratory, Ankeny, IA 50023

**Learning Overview:** After attending this presentation, attendees will better understand the impact of kava on Driving Under the Influence of Drugs (DUID) cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by strengthening the evidence that chronic or larger dosages of kava have detrimental effects on driving performance. To date, few reports investigate the impact of recreational use of kava on the cognitive and motor skills related to driving ability.

Kava, or *Piper methysticum*, is an Oceanic plant that is often consumed as a beverage. When consumed in therapeutic doses, kava has anti-anxiety effects. However, when consumed at high dosages, kava has been reported to produce sedation, tremors, and even a few cases of choreoathetosis. According to drug forums, recreational use of kava gives users a feeling of euphoria and heavy limbs. Despite being native to the Pacific islands, kava is becoming more and more popular in the United States, with kava bars popping up in various cities throughout the country. Kava teas, capsules, and powders are available for purchase at supermarkets and drug stores. Reports have shown that there does not appear to be significant impairment on cognition at acute, therapeutic doses of kava. However, there is evidence of the impairment of motor coordination and visual attention at larger chronic doses.

The psychoactive properties of kava have been attributed to lactone molecules primarily found within the root. Six kavalactones contribute to most of the pharmacological activity: kavain, 7,8-dihydrokavain, methysticin, 7,8-dihydromethysticin, yangonin, and desmethoxyyangonin. Many different modes of action have been suggested for the kavalactones. The main modes of action reported are the interaction of kavain on Gamma-Aminobutyric Acid (GABA) receptors, the interaction of yangonin on cannabinoid receptors, and blockage of voltage-gated Na<sup>+</sup> and Ca<sup>2+</sup> ion channels. It is these interactions that result in an effect similar to that of Central Nervous System (CNS) depressants and cannabis.

The Iowa Division of Criminal Investigation (DCI) Criminalistics Laboratory examined the data collected from suspected kava-impaired drivers. Four cases between November 2011 and April 2018 were reported by Drug Recognition Experts (DREs) for suspected kava use. Of the four cases, two urine samples were positive by Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) for the kavalactones yangonin and methysticin. Ethanol was detected in one case, but at <0.02g/dL. No blood samples were available for testing. DRE reports were provided for all four cases. Each of the four drivers admitted to drinking kava prior to driving and no other drugs were found during the toxicology analysis. In all four cases, DRE reports revealed that the kava-inhibited drivers did not pass the standardized field sobriety tests, and horizontal gaze nystagmus was present. The DRE officers in each case deemed the driver under the influence of a CNS depressant and unable to safely operate a motor vehicle. Until recently, no quantitative method was available in this laboratory for kavalactones. However, a new quantitative method was developed for yangonin and methysticin in urine by LC/MS/MS. The administrative cutoff was set to 10ng/mL, which was above the limit of quantification for both yangonin and methysticin in this study. The most recent kava case was analyzed with the new method, but significant degradation of kavalactones was noted, indicating possible instability within a urine sample. Analysis of the DRE and toxicology reports suggest that kava has an impairing effect on the motor skills required for driving.

#### Kava, Drug Recognition Expert (DRE), DUID



## K19 A Comparison of Multiple Extraction/Purification Methods for Novel Psychoactive Substances (NPS) From Biological Matrices

Ashley N. Kimble, BS\*, Miami, FL 33172; Anthony P. DeCaprio, PhD, Florida International University, International Forensic Research Institute, Miami, FL 33199

**Learning Overview:** The goal of this presentation is to demonstrate the results of comparing multiple extraction/purification methods for NPS in biological fluids (urine and whole blood).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by optimizing an extraction technique that is capable of handling high throughput and NPS of many different classes, which could greatly benefit forensic toxicology laboratories.

This research was designed to develop/optimize an extraction method capable of isolating a wide variety of NPS from biological fluids. This was accomplished by comparing multiple extraction methods, including online Solid Phase Extraction (SPE), classical SPE, crash/dilute and shoot, and (Quick, Easy, Cheap, Effective, Rugged, and Safe) QuEChERS. The focus of this work was to statistically compare the extraction/purification methods based on drug recovery, drug retention, reproducibility, minimization of matrix effects, time, and overall cost. Although extraction techniques for common drugs of abuse are well studied, developing extraction methods specifically targeting NPS is needed due to the increasing prevalence of NPS in forensic casework.

Dilute-and-shoot was accomplished by diluting urine samples with High-Performance Liquid Chromatography (HPLC) water using a 1:5 dilution followed by Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) analysis. For crash-and-shoot, 600 $\mu$ L of cold acetonitrile (-20°C) was added to 200 $\mu$ L of whole blood, vortexed, and centrifuged for 5min at 7,000rpm. After being centrifuged, the supernatant was removed and added to an LC vial, evaporated to dryness under a gentle flow of nitrogen, then reconstituted in 200 $\mu$ L of methanol for analysis. Classical SPE was performed following a method previously developed in the laboratory using a positive-pressure SPE apparatus and Agilent® Bond Elut Plexa PCX cartridges. Online SPE utilized an Agilent® 1290 Flex Cube LC unit with a Bond Elut (BE) online polymeric sorbent material (PLRP-S) cartridge.

QuEChERS has been shown to be a successful technique for extracting common drugs of abuse from biological fluids, but also has potential for extracting NPS. QuEChERS is an appealing alternative due to fewer transfer steps, quick extraction time, and decreased cost when compared to other approaches. This work used an in-house developed mini one-pot QuEChERS kit. Samples were added to pre-weighed components (MgSO<sub>4</sub>, NaCl, PSA, C18), shaken by hand, vortexed, and centrifuged at 7,000 rpm for 5min. After centrifugation, the acetonitrile layer was removed and added to an LC vial, dried, and reconstituted in 200 $\mu$ L of methanol. An Agilent® 1290 Infinity® HPLC system and Agilent® 6460 QqQ/MS with Jet Stream Technology Electrospray Ionization (ESI) operated in positive mode was used for analysis, along with an Agilent® Zorbax® Rapid Resolution HD Eclipse Plus™ C18 column for chromatographic separation. All samples were analyzed using a triggered Multiple Reaction Monitoring (tMRM) method that is validated for the detection of multiple NPS. All methods were tested using a mix of 36 NPS, which included compounds and metabolites from different drug classes, at three different concentrations (5ng/mL, 20ng/mL, and 80ng/mL). The four different extraction procedures for NPS in blood and urine were evaluated and compared using two-way Analysis Of Variance (ANOVA) to assess significant differences. If the ANOVA showed a significant difference, a Tukey Honest Significant Difference (HSD) test was completed to determine which specific methods were significantly different.

Findings demonstrated that NPS recoveries from urine with QuEChERS and dilute-and-shoot were not statistically different, while recoveries from blood were significantly higher with QuEChERS than with crash-and-shoot. For example, most synthetic cannabinoids demonstrated recoveries from urine above 75% with QuEChERS. QuEChERS, when used for both blood and urine, showed a decrease in matrix effects for all classes of NPS when compared to crash/dilute-and-shoot. While online SPE is an efficient option, it provided low recoveries for many classes of NPS, especially synthetic cannabinoids. Total time required from extraction start to analysis varied from 5min (dilute-and-shoot) to 3h (classical SPE), while costs varied from relatively inexpensive (dilute-and-shoot and QuEChERS) to expensive (online SPE and classical SPE, due to instrumentation and consumable costs, respectively). Although dilute-and-shoot was the quickest and most cost effective, it is a crude method that can leave matrix components, which can damage instrumentation and lead to unwanted ion suppression and enhancement. In conclusion, results showed that QuEChERS provided the best combination of extraction capability, elimination of matrix effects, time, and cost for application to NPS analysis.

### Extraction Methods, LC/QqQ/MS, Novel Psychoactive Substances



### **K20 Drunk Driving Detected by an Expiratory Alcohol Detector After Chewing *Areca Catechu*: A Case Report and Result Analysis**

*Jingjie Wu, PhD\**, Institute of Forensic Science, Guizhou Police, Guiyang 550005, CHINA

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**THIS ABSTRACT WAS NOT PRESENTED.**





### K21 The Detection and Quantification of 23 Drugs in Blood and Urine Following Solid Phase Extraction (SPE) Using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS)

Courtney McGowan, BS\*, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Kelsie L. Jenquine, BS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Michael Moretto, BS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118; Shawn Foley, BS, Boston University, Biology Department, Boston, MA 02118; Nichole D. Bynum, MS, RTI International, Johnson Building, Research Triangle Park, NC 27709; Katherine N. Moore, MS, Research Triangle Park, NC 27709; Sabra R. Botch-Jones, MS, Boston University School of Medicine, Biomedical Forensic Sciences, Boston, MA 02118

**Learning Overview:** After attending this presentation, attendees will be able to use the method presented, or develop their own singular method, for both blood and urine analysis of selected amphetamines, antidepressants, anesthetics, opioids, designer, and hallucinogenic drugs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by offering a method that will provide the ability to analyze different drug groups in one method for both blood and urine matrices.

**Background/Introduction:** In forensic toxicology, analysis of drugs in biological fluids is performed to determine cause of death, suspected drug use, drug-facilitated crimes, or whether someone was driving under the influence. Determining what analytes are present and the concentration of those compounds in a variety of matrices (e.g., blood, urine, or oral fluid) can be complex. It is therefore necessary to have optimal sample preparation and instrumental conditions that work for all matrices of interests. Determining the best approach can be challenging due to the amount of time and resources to perform expansive evaluations of sample preparation, stationary/mobile phases, LC conditions, and MS operating parameters.

**Objective:** This project developed and validated an SPE and UPLC-MS/MS method using both human whole blood and urine to identify and quantify selected amphetamines, antidepressants, opioids, anesthetics, designer drugs, and hallucinogenic drugs.

**Method:** Calibration curves and Quality Controls (QCs) were prepared for SPE in 200 $\mu$ L of either drug-free whole blood or donated urine. All donated samples were collected following approved Institutional Review Board requirements. Analytes were spiked at varying concentrations using certified reference standards. Deuterated internal standards were also spiked at a concentration of 200ng/mL. QCs were prepared at 20ng/mL, 125ng/mL, 450ng/mL, and 950ng/mL. SPE was performed with mixed-mode copolymeric Clean Screen<sup>®</sup> DAU columns. Samples were reconstituted in 400 $\mu$ L of Millipore<sup>®</sup> water containing 0.1% formic acid.

The samples were run by UPLC with a 4000 QTRAP<sup>®</sup> Electrospray Ionization Tandem Mass Spectrometry (ESI/MS/MS) in positive ionization mode. Separation was achieved using a Kinetex<sup>®</sup> F5 2.6 $\mu$  100 $\text{Å}$  50mm x 3.0mm column.

**Results:** Bias, precision, Limit Of Detection (LOD), Limit Of Quantitation (LOQ), calibration model, carryover, and dilution integrity, ion suppression and enhancement, and processed stability validation parameters were assessed. All analytes in both matrices had quadratic fit with 1/x weighting, LOQs were 0.5ng/mL, 5ng/mL or 10ng/mL, and no observed carryover. Calibration ranges of 10ng/mL to 1,000ng/mL, 0.5ng/mL to 1,000ng/mL, and 0.5ng/mL–500ng/mL were used. For the 23 drugs in blood, the bias ranged from -18.10% to 11.87%, precision ranged from 0.24% to 14.33%, and recovery in matrix ranged from 85% to 119%. In urine, the bias ranged from -5.40% to 7.67%, precision ranged from 0.09% to 11.99%, and recovery in matrix ranged from 80% to 119%.

**Conclusion/Discussion:** The method containing 23 drugs, including selected amphetamines, antidepressants, anesthetics, opioids, designer, and hallucinogenic drugs, was successfully validated in both blood and urine using the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for method validation. SPE provided clean extracts for analysis by positive ESI-UPLC-MS/MS for both matrices.

#### Solid Phase Extraction, Forensic Toxicology, Multi-Drug Analysis

## K22 Driving Under the Influence of Drugs (DUID) in Different Countries: An Overview

Aldo Di Nunzio\*, Magna Graecia University, Viale Europa, Germaneto, Catanzaro 88100, ITALY; Michele Di Nunzio, BS\*, Biogem scarl, Via Camporeale, area PIP, Ariano Irpino (AV) 83310, ITALY; Antonello Crisci, MD, University of Salerno, Salerno 84084, ITALY; Ciro Di Nunzio, PhD\*, Magna Graecia University Legal Medicine, Viale Europa, Germaneto, Forensic Genetics Laboratory, Catanzaro 88100, ITALY

**Learning Overview:** After attending this presentation, attendees will understand different countries' policies regarding DUID and how they differentiate from Driving Under the Influence of Alcohol (DUIA).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how different nations/states are fighting the issue of DUID and the importance of synergistic cooperation between police, medical professionals, and forensic toxicologists.

Driving a motor vehicle is not easy. It is a skills-required task and drivers should be aware of their own safety and that of others. Hence, taking substances, such as alcohol or other drugs (also prescribed), before driving is a bad idea. The effects of alcohol on performance and behavior and the clinical tests for drunkenness are widely documented. Indeed, blood alcohol concentration—set as 0.5g/L—is a value internationally recognized by most countries. Studies show that a person's demeanor is altered at this concentration.<sup>1</sup> On the other hand, the situation for drugs is deeply different. While alcohol is just one molecule, drugs represent a class of molecules, totally different from each other (depressants, stimulants, hallucinogens, etc.). Moreover, there are different responses, inter-individuals and intra-individuals, to drugs. Some “drug naïve” subjects can experience dissimilar feelings from drug addicts.<sup>4</sup> Therefore, in this complicated scenario, an arbitrary drug level cannot be set or, at least, driving impairment cannot be defined only on “a laboratory outcome.” There are two main approaches to legislating against drivers who drive while under the influence of drugs. The first is an impairment standard, under which a driver is guilty of the crime of Driving Under the Influence (DUI) if it can be shown that their driving ability is affected by drug or alcohol use. This is the so-called “affected by” approach and it is the most straightforward and relates the person's behavior to the crime of impaired driving. Evidence to support these charges may include appearance, speech, divided attention and balance, etc. The second approach to prosecuting impaired driving behavior is the so-called “per se approach.” Under this construction, the government, based on its obligation to preserve public health and welfare and in consideration of the risks to its citizens of sharing the roadways with impaired drivers, has moved to outlaw driving after having consumed a drug with potentially intoxicating properties. This approach is called per se if a quantitative standard in blood, oral fluid, or urine is set above which driving is prohibited.<sup>2</sup> Today, countries/states are adopting different policies. In Europe, only a few nations (Belgium, Czech Republic, Republic of Ireland, Luxembourg, Norway, United Kingdom) out of 26 set limit values for some drugs (THC, amphetamines, cocaine, morphine).<sup>5</sup> By contrast, in the United States, Nevada and Ohio show a straightforward law, where limits were set also for drugs' metabolites.<sup>3</sup> Other countries (Australia and Russia) have a “zero tolerance;” they did put the limits at the cut-off levels. Italy, as other countries (Lithuania, Malta, etc.), has established no drug limits. In these cases, the offended impairment level is defined when the drug is detected in the blood. Furthermore, it is important for countries/states with no per se law (Alabama, New Jersey, Argentina, etc.) to train police officers to recognize impaired drivers throughout several tests such as a walk-and-turn test, a one-legged-stand, and the assessment of Horizontal Gaze Nystagmus (HGN).

In conclusion, it is significant to understand how drugs affect individual components of the task of driving and how they can affect their overall performance. To consider the extent of the impairment, an overall consideration about behavioral domain analysis, epidemiological monitoring, and empirical toxicological assessment should be performed. To achieve this, a synergistic cooperation among police officers, medical professionals, and forensic toxicologists is extremely important.

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### Forensic Science, Driving Impairment, Drugs



### K23 The Impact of Storage Conditions, Sample Volume, and Collection Technique on Blood Alcohol Concentration (BAC) in Non-Decomposed Defibrinated Sheep's Blood

Christian T. Pascual\*, Emporia State University, Emporia, KS 66801; S. Sharee Lambert, BA, Emporia State University, Emporia, KS 66801; Carrie Hodges, MLS, Kansas Bureau of Investigation, Topeka, KS 66604; Melissa M. Bailey, PhD, Emporia State University, Emporia, KS 66801

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the effects of the collection technique, time, temperature, sample volume, and presence of excess glucose on the BAC in non-decomposed whole blood.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how the percentage of ethanol is affected when the blood sample is collected and stored under various conditions over a five-month period. This study also investigated how the presence or absence of excess glucose (>240mg/dL) affects the BAC over time, given that blood is often collected from suspects or victims with diabetes or metabolic disorders. The results of this longitudinal study could springboard to policy reforms in terms of the process of collection, storage, handling, or testing of samples taken from suspects of Driving Under the Influence (DUI) cases.

The forensic science community worldwide has no standardized procedures for the collection, storage, handling, or testing of samples for blood alcohol analysis. Degradation of ethanol in blood alcohol samples can be caused by storage temperature, time of storage, and sample volume.<sup>1</sup> If samples need to be re-analyzed or if significant time elapses between evidence receipt and analysis, the samples must be stored correctly to ensure accurate results.<sup>2</sup>

Two sets of aliquots of seven different ethanol concentrations were prepared in defibrinated sheep blood (Hemostat Laboratories): 0g/dL, 0.05g/dL, 0.08g/dL, 0.10g/dL, 0.15g/dL, 0.20g/dL, and 0.30g/dL. D-glucose from Fisher Scientific was added to one set of aliquots in sufficient quantity to result in a blood glucose measurement of at least 240mg/dL. The appropriate sample from each of the aliquot was then added to 10mL gray-stoppered BD Vacutainer® blood collection tubes (GST) in varying amounts: 2.5mL, 5mL, 7.5mL, and 10mL, either by drawing under vacuum (method 1) or by removing the stopper and adding the blood via syringe (method 2). For each ethanol concentration, four groups of samples were made. Group 1 had tubes of each volume as described, with and without excess glucose, collected using method 1 and stored at room temperature (25°C)—8 tubes in total. Group 2 had 8 tubes as in group 1 but were refrigerated at 4°C. Group 3 had 8 tubes as in group 1 but collected using method 2 and stored at room temperature. Group 4 had 8 tubes as described in group 3 but refrigerated at 4°C. All four groups were made at each BAC for monthly analysis (months 0–5), for a total of 1,344 tubes. All the tubes were aliquoted at the beginning of the study. Room temperature was chosen to study the effects of improper storage conditions that could be encountered if samples are not appropriately refrigerated in a timely manner. Each month, samples were analyzed in duplicate with an internal standard of 0.002% 1-propanol in water by an Agilent® 7820A GC and 5977E MS with headspace after instrument calibration; with 0.10g/dL standards run every 46 vials.

The data in the study were analyzed using Analysis of Variance (ANOVA) in which the level of significance was tested with the *p*-value of 0.05. The study discovered that over time, samples with lower volume had a greater ethanol loss than samples with higher volume (0.014g/dL vs 0.006g/dL). Additionally, the samples collected through method 1 had more ethanol loss than those collected through method 2 (0.01g/dL vs 0.008g/dL). Samples that were stored at room temperature had a higher percentage of ethanol loss as compared to those that were refrigerated (0.016g/dL vs 0.004g/dL). However, the presence of excess glucose does not significantly affect ethanol loss. These findings show that storage temperature, collection technique, and sample volume may affect ethanol concentration in blood samples and these factors should be considered when drawing conclusions about data obtained from case samples.

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#### BAC, Collection Technique, Storage Condition



## K24 *In Silico* Simulation of Fentanyl Raman Spectra

Madison R. Schackmuth, BS\*, Huntsville, TX 77340; Patrick Buzzini, PhD, Sam Houston State University, Huntsville, TX 77340; Darren L. Williams, PhD, Sam Houston State University, Huntsville, TX 77341

**Learning Overview:** After attending this presentation, attendees will be familiar with the use of Gaussian modeling for the simulation of fentanyl Raman spectra.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by encouraging preemptive characterization of novel fentanyl structures with Gaussian modeling of Raman spectra.

Fentanyl and its analogs (fentologs) are mu ( $\mu$ ) receptor agonists capable of causing severe respiratory depression in conjunction with the typical opioid toxidrome.<sup>1</sup> The number of deaths associated with fentanyl and its analogs has increased, particularly in North America, and cases involving seized fentologs more than doubled from 2015 to 2016.<sup>2,3</sup> Potential hazards to law enforcement, emergency responders, and forensic scientists have also drawn concern. Screening methods that can keep pace with the fentolog development are in high demand. Raman spectroscopy is non-destructive and capable of elucidating chemical structures of substances through packaging in solution, in all states of matter.<sup>4</sup> Due to safety concerns and licensure requirements for obtaining powdered forms of Schedule I controlled substances, modeling of fentolog structures was performed *in silico*. Predictive modeling of Raman spectra can be used to explore the properties of novel synthetic structures and create libraries prior to their appearance on the illicit drug market.

Gaussian 09 software package and density functional theory calculations were used to simulate Raman spectra for the fentologs. The fentanyl structure was modeled in GaussView 5.0 software. The geometry of the structure was optimized, and the vibrational frequencies determined through simulation with the Gaussian 09W software. Four levels of theory were used to optimize the geometry including molecular mechanics, semi-empirical, Hartree-Fock (HF), and Density Functional Theory (DFT). The simulation excluded solvation as a parameter and was optimized at room temperature. Fentanyl was optimized at a vibration theory/geometry optimization of B3LYP/6-311G(d)/B3LYP/6311G(d). The structures of seven fentologs ((+)-Cis-3-methylfentanyl, 4-ANPP, acetylfentanyl, butyrylfentanyl, furanylfentanyl, isobutyrylfentanyl, and norfentanyl) were modified in GaussView 5.0 from the optimized fentanyl structure. The analogs were simulated through three levels of theory and basis sets: HF STO-3G, DFT B3LYP 6-31G(d), and DFT B3LYP 6-311G(d). The total energies (Hartree) of the simulated structures were compared to assist in selecting the lowest energy structure. The transition wavenumbers and intensities calculated by Gaussian were plotted in a spreadsheet. A normal distribution was plotted around each transition wavenumber (spectral line) considering the spectral resolution (transition width) as the standard deviation. The normal distribution was multiplied by the transition intensity. The area under the normal distribution curve at each increment wavenumber value along the x-axis was summed to graph the simulated intensity. Elevated baseline and baseline slope parameters were simulated by adding a user-defined value to the spectral intensities. Spectral resolution was administratively set at 8  $\text{cm}^{-1}$ . The fentolog spectra are consistent across modification groups with the presence or absence of bands corresponding to specific vibrations that can be utilized for differentiation.

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### Raman Spectroscopy, Fentologs, Gaussian



**K25 The Development of a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Analytical Method for Simultaneous Detection of Tetrahydrocannabinol (THC), Cannabidiol, Cannabinol, and 11-Nor-9-Carboxy-Tetrahydrocannabinol in Hair and Its Application to Authentic Hair Samples**

*Byungseok Cho, PhD, National Forensic Service, Wonju 26460, SOUTH KOREA; Han Soo Cho, MD, National Forensic Service, Wonju 26460, SOUTH KOREA; Sangwhan In, PhD, National Forensic Service, Wonju 26460, SOUTH KOREA; Eunmi Kim, PhD\*, National Forensic Service, Wonju, Gangwon-do, Wonju, SOUTH KOREA*

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**THIS ABSTRACT WAS NOT PRESENTED.**

### K26 The Great Enigma of the Cause of Death in Burns: The Role of Forensic Toxicological Surveys—A Case Report and Review of the Literature

Isabella Aquila, MD\*, Institute of Legal Medicine, University Magna Graecia of Catanzaro, S Venuta-Medicina Legale, viale Europa, Catanzaro 88100, ITALY; Santo Gratteri, MD, Viale Europa, Germaneto, Catanzaro 88100, ITALY; Roberto Raffaele, BE\*, University Magna Graecia of Catanzaro, Via Thailandia N 1, Crotona, ITALY; Fabrizio Cordasco, MD, Università Magna Graecia CZ, Viale Europa, 88100, Catanzaro, ITALY; Francesco Sicilia, MD\*, Institute of Legal Medicine, Viale Europa, 88100, Catanzaro, ITALY; Matteo A. Sacco, MD\*, Chair of Legal Medicine, University of Catanzaro, Viale Europa, Loc Germaneto, Catanzaro 88100, ITALY; Pietro Tarzia, MD, Institute of Legal Medicine, Viale Europa, 88100, Catanzaro, ITALY; Simona Mongiardi, Institute of Legal Medicine, Viale Europa, 88100, Catanzaro, ITALY; Pietrantonio Ricci, MD, PhD, Department of Clinical and Experimental Medicine, Section of Legal Medicine, University of Foggia, Foggia 71100, ITALY

**Learning Overview:** After attending this presentation, attendees will be able to show how the toxicological examination remains a crucial scientific piece of evidence in cases of death from thermal energy, especially when the autopsy does not clarify all aspects.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by emphasizing the role of toxicological investigation and the circumstantial data in order to investigate the correct sequence of events in a fire.

Traumatic injuries due to thermal energy include all the effects produced by exposure of the human body to temperatures that are too high or too low compared to the body's adaptability. The type of lesions sustained depends on the temperature level. Heat injuries can be determined by the increase in temperature, electricity, radiation, nuclear energy, or corrosive or caustic substances. The contact between a heat source and the body surface determines the production of burns on the skin. In some cases, the affected area is large, so that in addition to local effects, a systemic syndrome occurs that affects various organs and systems. Burns are a serious public health problem. Burns generally occur in domestic or work environments, but also as a result of traffic accidents, self-harm, or assaults.<sup>1</sup> Burns are classified, according to the depth of the lesion, as: first degree—epidermis; second degree—superficial dermis; third degree—deep dermis; fourth degree—deep soft tissues; and fifth degree—bones and joints.<sup>2,3</sup>

The analysis of thermal energy lesions is fundamental to establish the therapy and the prognosis for the patient. In the forensic field, the main purpose is understanding the cause and the modality of death. The injuries can be caused by a murder, an accident, or a suicide. In cases of death related to a fire, the autopsy is not always sufficient to establish the diagnosis and the manner of death. This study will demonstrate the determinant contribution of toxicological analysis in the diagnosis and reconstruction of the mode of death, in accordance with other data collected.

Reported here is the case of two boys found dead inside a commercial premise destroyed by fire. At the entrance to the room, it was noted that the interior had been destroyed by the flames, with evidence of an intense smell of flammable liquid. The 3D Computed Tomography (CT) excluded the presence of gunshots in both cases. In the first case, the autopsy showed deep burns, especially on the arms and legs, with black smoke spots on the palate. In the second case, superficial burns and the presence of soot in the glottis were detected. The percentage of carboxyhemoglobin and the presence of alcohol, drugs, or psychoactive drugs by mass spectrometry were investigated. The toxicological analysis showed in both cases that death was due to carbon monoxide poisoning in different percentages: 40.75% in the first case; 24.18% with presence of ecgonine and benzoylecgonine in the urine in the second case. In the first case, death was also due to burns spread by burning highly inflammable material.

The analysis of the cause of death in victims of fires is a great forensic enigma. In these cases, death may have occurred before, during, or after the fire. To reconstruct the event, the forensic pathologist must evaluate the circumstantial data during the inspection and investigate the signs of vitality at autopsy. Among these is highlighted the discovery of the soot in the glottis, in the trachea, and in the lungs, with the presence of extensive burns on the corpse. Often the signs present at the autopsy are not sufficient for diagnosis. In literature, a retrospective study on 107 firefighters showed that only about 65% of cases show signs of viability in the airways at autopsy. In the remaining cases, the signs are not significant or are even absent.<sup>4</sup> In these events, the role of toxicological investigation is crucial. Carbon monoxide levels are indicative that the victim was still alive at the time of the fire.<sup>4-7</sup> In this case, the toxicological data has been decisive for determining the cause of death, but has also contributed to defining the modalities of the event. This investigation, in agreement with the circumstantial data, suggested that the disaster had been caused by arson. The performing of 3D CT and autopsy remains essential to rule out other possible causes of death.<sup>8</sup>

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**Forensic Science, Burns, Carboxyhemoglobin**

### K27 Metabolic Profiling of the Synthetic Cannabinoid AB-FUBINACA Using an Electrochemical Cell, Human Liver Microsomes, Cryopreserved Hepatocytes, and Liquid Chromatography/High Resolution Mass Spectrometry (LC/HRMS)

Eduardo M. Cardoso, MSc\*, Federal Police of Brazil, Minas Gerais 30441-170, BRAZIL

**Learning Overview:** After attending this presentation, attendees will better understand the challenges associated with Synthetic Cannabinoids (SCs) analyses and the different approaches to tackle the problem. The goal of this presentation is to assess electrochemical cell forced oxidation as a suitable tool for metabolite investigation and to identify major and specific biomarkers to unequivocally confirm intake of the synthetic cannabinoid AB-FUBINACA.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by presenting an unusual tool as a complementary technique for the characterization of metabolic pathways of new chemical entities.

In the present research, the goal is to characterize the phase I metabolism of the SC AB-FUBINACA, chosen due to recent prevalence studies.<sup>1</sup> *In vitro* metabolism was investigated using Human Liver Microsomes (HLM), Cryopreserved Human Hepatocytes (CHH) and forced electrochemical oxidation assays, and the metabolic entities separated and identified using LC/HRMS.

One of the main issues of SC analysis is the emergence of new compounds with unknown metabolic profiles, which means no urinary marker metabolites are known. Since the clandestine chemists' strategy is to make minor alterations on the pharmacophore core of the molecule, it is not uncommon to have the same metabolites coming from different parent SCs. For instance, 5'-OH-JWH-018 can derive from hydroxylation of the JWH-018 pentyl chain, but also from oxidative defluorination of AM-2201.<sup>2</sup> This is even more problematic when isomeric compounds are present with similar metabolic pathways, with one drug scheduled while its pair is not, such as the case with BIM-2201 (FUBIMINA) and THJ-2201 (each scheduled in the United States on different dates).<sup>3</sup>

With the data gathered in this research, a metabolic pathway was proposed. The incubation of AB-FUBINACA with HLMs in the presence of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) resulted in the formation of six metabolites, including two mono-hydroxylations (M1.1 and M1.2), the amide hydrolysis (M2), a dehydrogenation (M3), and two mono-hydroxylations of the amide hydrolysis (M4.1 and M4.2). The hepatocytes yielded similar results, but the prevalence of the metabolites changed, being mainly dominated by the amide hydrolysis and some related by-products. The Electrochemical Cell (EC) -forced oxidation displayed a range of multiple hydroxylations (mono, di, and tri), carbonylations/ epoxylation, and carboxylations, although no amide hydrolysis could be detected. Authentic urine samples confirmed that the hepatocytes displayed the closest correlation between *in vivo* and *in vitro* investigations. The electrochemical cell-forced oxidation proved to be a fast and cost-effective tool for the prediction of new chemical entities. However, it was not able to predict the main *in vivo* metabolic pathway, probably due to the conditions used in the experiment (low buffer strength, room temperature, and duration of the assay). Optimization of the EC assay conditions may lead to better results.

It seems that the amide hydrolysis (M2) and the glucuronidation of the amide hydrolysis (M5) are suitable urinary markers, but these are not specific markers for intake of AB-FUBINACA, since they can be formed by ester hydrolysis from AMB-FUBINACA or EMB-FUBINACA. A better biomarker would be a metabolite with intact terminal carboxamide groups, like M1.2, but it was only found in the *in vitro* experiments and not in the urine samples. Further *in vivo* investigations are suggested to confirm or refute M1.2 as a suitable marker and evaluate the influence of inter-individual variability.

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#### *In Vitro* Metabolism, Electrochemical Cell, Synthetic Cannabinoids





## K28 Methadone Overdose in Patients Following Methadone Maintenance Treatment: An Italian Issue

Matteo Favia\*, Laboratory of Forensic Toxicology, Bari 70124, ITALY; Ilaria Santoemma, Laboratory of Forensic Toxicology, Bari 70124, ITALY; Quintino Mita, BS, Laboratory of Forensic Toxicology, Bari 70124, ITALY; Giuseppe Strisciullo, BES, University of Bari, Bari 70124, ITALY; Francesco Introna, MD, Dim Sezione Di Medicina Legale, Bari 70124, ITALY; Antonio De Donno, PhD, Laboratory of Forensic Toxicology, Bari 70124, ITALY

**Learning Overview:** The goal of this presentation is to increase awareness of the problem of overdose deaths in patients following Methadone Maintenance Treatment (MMT) in Italy.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by underlining the importance of developing new guidelines in methadone maintenance treatment for opioid-addicted patients in order to avoid overdose deaths.

Methadone is an opioid like heroin or opium. MMT is recognized as a reference treatment for opioid dependence. Cases of methadone overdose in patients following MMT are already described in the existing scientific literature.

According to Italian law, at the beginning of the treatment, patients must receive medication under the supervision of a physician to avoid an overdose. After a period of stability, patients are allowed to take methadone at home in pre-arranged and personalized concentrations to empower their self-responsibility.

It has been estimated that plasma methadone levels should be at least 0.05mg/L–0.10mg/L (50ng/ml–100ng/ml) to prevent withdrawal symptoms in narcotic maintenance patients.

In methadone maintenance subjects, the mean blood level of methadone is around 110ng/ml. Detected average concentration of methadone was 280ng/ml in 59 victims of fatal methadone overdose. Methadone is largely metabolized by mono and di-N-demethylation, with spontaneous cyclization of the resulting unstable metabolites to form 2-ethylidene-1,2-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenylpyrrolidine (EMDP). In some cases, it may be useful to quantitate EDDP concentrations, as the presence of the metabolite in substantial amounts may indicate prior usage of the drug and therefore tolerance to its effects, although the organs' relative amounts of methadone and EDDP would of course also depend on survival times after administration.

In the past year, five forensic autopsies on corpses of regular opioid users were performed at the Institute of Legal Medicine of Bari, Italy. Four were found dead in their homes. In two of those cases, there were empty and loaded syringes next to their bodies and multiple track marks over the bodies. In one case, the subject died from a stabbing, but she was following MMT.

In all these cases, very high methadone concentrations in blood were found (785ng/ml, 1,356ng/ml, 1,188ng/ml, 626ng/ml, 983ng/ml). Toxicological analysis also showed very high concentrations of EDDP in blood (respectively 1,494ng/ml, 2,790ng/ml, 1,565ng/ml, 1,23 ng/ml, 490ng/ml). The average blood level of methadone in these cases was 987.6ng/ml, against 1,514.4ng/ml of EDDP. Both Methadone (MTD) and its metabolite 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) were identified and quantified by Gas Chromatography/Mass Spectrometry (GC/MS) analysis on an Agilent® 6890 GC coupled 5973 inert mass spectrometer, following Liquid-Liquid Extraction (LLE) from cadaveric blood by organic mixture solvents at pH 9.0. The amount of sample for analysis was 1ml.

Overdose with methadone is characterized by stupor, muscle flaccidity, respiratory depression, cold and clammy skin, miosis, hypotension, coma, and circulatory collapse. After performing an autopsy and ancillary analysis, it was possible to conclude that the cause of death was respiratory depression by overdose of methadone in four cases. In the cases described in this study, no other substance of abuse was found, apart from the fifth case that showed both methadone and cocaine abuse. All five subjects were following an MMT in a public health institute.

Fatalities in adults from methadone overdose have increased significantly in many urban areas because of the widespread availability of the drug, both from licit and illicit sources. An improvement of the MMT's guideline is needed to reduce future methadone overdose deaths. This study suggests delivering methadone under strict medical control, not only to avoid cases of overdose, but also the possibility that opioid users following MMT are selling methadone on the black market. A strict monitoring of the drug concentration in the blood of the addict attending MMT to assess adherence to the medical plan is also recommended.

### Methadone, Overdose, Methadone Maintenance Treatment



## K29 Yohimbine Quantification in Postmortem Specimens: Two Case Reports

Matthew N. Newmeyer, PhD\*, Office of the Chief Medical Examiner, Baltimore, MD 21223; Rebecca Jufer Phipps, PhD, Office of the Chief Medical Examiner, Baltimore, MD 21223; Barry S. Levine, PhD, Office of the Chief Medical Examiner, Baltimore, MD 21223; Zabiullah Ali, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223; John Stash, DO, Office of the Chief Medical Examiner, Baltimore, MD 21223; David R. Fowler, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223

**Learning Overview:** The goal of this presentation is to describe two postmortem cases in which yohimbine was identified during routine analysis.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by adding valuable data for improved interpretation of yohimbine-positive postmortem cases.

Yohimbine is an indole alkaloid, approved in the United States to treat erectile dysfunction. It is also abused on the streets and used as a nutritional supplement among body builders.<sup>1</sup> Yohimbine is a potent  $\alpha$ -adrenoreceptor antagonist, leading to an increase in noradrenaline and dopamine.<sup>2</sup> Yohimbine was identified in two postmortem cases during routine analysis and quantified via a validated Gas Chromatography/Nitrogen Phosphorus Detection (GC/NPD) method.

In the first case, the decedent was a 53-year-old male found alone in his residence. The decedent had no significant medical history; however, he was a known Phencyclidine (PCP), cannabis, and ethanol user. Heart blood, subclavian blood, urine, bile, liver, and kidney were submitted for toxicological analysis. The second case involved a 47-year-old male who was reported missing. The decedent's car was located parked on a bridge, and the decedent was later found 2,000 yards from shore. According to the decedent's mother, the decedent was depressed with previous suicide attempts. He was a known methadone and cocaine user. Cavity blood, urine, bile, liver, and kidney were submitted for analysis. Routine toxicological analysis included volatiles, an acidic/neutral drug screen, an alkaline drug screen, and Enzyme-Linked Immuno-Sorbent Assay (ELISA) for morphine, benzodiazepines, and oxymorphone.

In case 1, the urine drug screen detected PCP and yohimbine; ethanol and other volatiles were not detected in the heart blood. PCP was quantified in the heart blood at 0.1mg/L. Yohimbine in the heart and subclavian blood were 7.3mg/L and 5.3mg/L, respectively (central/peripheral ratio 1.4); additionally, yohimbine in the urine, bile, liver, and kidney were 34mg/L, 11mg/L, 47mg/kg, and 10mg/kg, respectively (liver/central ratio 6.4, kidney/central ratio 1.4). The cause and manner of death were yohimbine intoxication and undetermined, respectively. In the second case, the urine drug screen was positive for several substances; on subsequent analysis, the cavity blood contained cocaine <0.05mg/L, benzoylecgonine, methadone 1.0mg/L (liver methadone 3.4mg/kg), dextromethorphan 0.1mg/L, and mirtazapine 0.06mg/L. Ethanol and other volatiles were not identified. Yohimbine was only identified in the cavity blood and urine at 0.1mg/L and 0.2mg/L, respectively. The cause and manner of death were multiple injuries and drowning and suicide, respectively.

There are few reported postmortem yohimbine data in the literature. In one report of two fatal overdoses, yohimbine was identified in iliac blood at 7.4mg/L (case 1) and heart blood at 5.4mg/L (case 2).<sup>3</sup> In another report, a patient presented to the hospital following ingestion of a large amount of yohimbine and was successfully treated; 3h after ingestion the blood yohimbine concentration was 5.2mg/L.<sup>1</sup> The central and peripheral blood yohimbine concentrations from case 1 presented agree with postmortem concentrations previously reported, while the cavity blood yohimbine concentration in case 2 falls in the range of maximum concentrations observed in controlled administration studies reported elsewhere.<sup>2</sup> Care must be taken when interpreting and comparing postmortem drug concentrations, and postmortem redistribution must always be considered. The data presented indicated yohimbine may be susceptible to redistribution. These data are a valuable addition to the toxicological literature and will help improve interpretation of postmortem yohimbine concentrations.

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### Yohimbine, Postmortem Toxicology, Overdose



### **K30 Validation of an Assay for Amphetamines in Postmortem Samples Using Supported Liquid Extraction (SLE) and Biotage® Extrahera™ Automation Followed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Analysis**

*Heidy P. Rivera, BA\*, Elizabeth, NJ 07202; Kristen M. Bailey, MS, Office of the Chief Medical Examiner, Charleston, WV 25302; Lauren L. Richards-Waugh, PhD, Marshall University Forensic Science Program, Huntington, WV 25701; James C. Kraner, PhD, Office of the Chief Medical Examiner, Charleston, WV 25302*

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the importance of integrating automation in the forensic toxicology laboratory for the purpose of reduced analyst time and improved assay consistency.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that the Biotage® Extrahera™ robotic platform for automated drug extraction is capable of efficiently and reliably processing up to 96 samples in a single batch. This method validation demonstrated that the automated method was capable of successfully performing extractions at the intended use level and can be used to replace the current manual extraction for casework involving amphetamines.

The Biotage® Extrahera™ is an automated robotic instrument that can perform an extraction on up to 96 samples, allowing for an increase in time efficiency and minimization of error by increasing pipetting precision and reducing the risk of contamination between samples. The Biotage® Extrahera™ utilizes an interchangeable platform that can be customized for SLE, SPE, Phospholipid Depletion (PLD), and Protein Precipitation (PPT)-based methods.

In this study, an SLE method for amphetamines in postmortem whole blood using the Biotage® Extrahera™ and LC/MS/MS analysis was validated in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology published in 2013. A 200µL aliquot of calibrators, controls, and whole blood samples were manually pipetted onto a Thomas Scientific 1mL, 96-well plate that was loaded onto the Biotage® Extrahera™ where the samples were extracted utilizing a Biotage® Isolute® SLE+ 400µL 96-well plate and a Waters® 2mL 96-well square collection plate. Deuterated analogs of the analytes of interest were used as internal standards. A 100µL aliquot of the eluate was evaporated to dryness and reconstituted on the Biotage® Extrahera™ with 1000µL of Optima-grade water for injection onto a Waters® LC/MS/MS system. Samples were then analyzed by electrospray ionization in positive-ion Multiple Reaction Monitoring mode with optimized collision energy for the precursor ion selected, monitoring two or three transitions for each analyte of interest. The validation plan consisted of studies for various interferences, calibration model, ionization suppression/enhancement, carryover, bias and precision, Limit Of Detection (LOD), and Limit Of Quantitation (LOQ). The analytes of interest were amphetamine, methamphetamine, phentermine, ephedrine/pseudoephedrine, phenylpropanolamine, MDA, and MDMA.

This method produced data that met the acceptance criteria established for the validation. The internal standard, analyte, and matrix interference studies revealed that no deuterated internal standard, commonly encountered non-target analytes, or matrix components produced a signal for any of the target analytes. The method was free from carryover at 3,000ng/mL for each analyte. Ionization suppression or enhancement was less than ±25%. The proper calibration model and weighting function was chosen for each analyte using a Microsoft® Excel® spreadsheet. The method also produced the following quantitative data: the LOD and LOQ for amphetamine, methamphetamine, phentermine, ephedrine, phenylpropanolamine, MDA, and MDMA were 20ng/mL, 10ng/mL, 40ng/mL, 10ng/mL, 10ng/mL, 10ng/mL, and 10ng/mL, respectively. For all compounds, bias and precision were within 20%.

#### **Automation, Amphetamines, LC/MS/MS**

### **K31 Method Validation for Simultaneous Identification and Quantification of Postmortem Volatiles in Whole Blood (WB), Vitreous Humor (VH), and Cerebrospinal Fluid (CSF) Using Gas Chromatography/Headspace/Flame Ionization Detector (GC/HS/FID)**

*Supriya Krishna, MSc\*, All India Institute of Medical Sciences, Department of Forensic Medicine & Toxicology, New Delhi 110092, INDIA; Om P. Murty, MD, All India Institute of Medical Sciences, Department of Forensic Medicine & Toxicology, New Delhi 110092, INDIA; Ashok K. Jaiswal, PhD, Forensic Medicine, New Delhi 110029, INDIA; Tabin Millo, MD, All India Institute of Medical Sciences, Department of Forensic Medicine & Toxicology, New Delhi 110092, INDIA*

**Learning Overview:** After attending this presentation, attendees will gain appreciation for the application of a validated GC/HS/FID methodology for simultaneous detection and quantification of seven volatiles as detected in stored whole blood, vitreous humor, and cerebrospinal fluid.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by helping toxicologists understand the intricacies and significance of analyzing many volatiles in single run using acetonitrile as an internal standard. This will also help attendees better understand the correlation of ethanol concentration between each body fluid.

Determination of blood alcohol content is a frequently requested assay in forensic toxicology laboratory. Although determination of alcohols in blood is a routinely used procedure, the results and methodologies are constantly improving. A highly sensitive, reproducible, and rapid GC method was validated for the analysis of seven volatiles frequently encountered in stored samples and samples collected within hours of autopsy. The extensive review of literature in this field yielded methods with extensive instrumentation and method preparation. Although the use of GC for the determination of blood alcohol concentration is a routinely used procedure, its validation for the use for stored and postmortem samples is an emerging research field. This presentation covers a method, using GC/HS/FID, capillary column (DB-624) and carrier gas-Nitrogen, validated to analyze ethanol, methanol, acetone, acetaldehyde, N-propanol, I-propanol, and N-butanol in different postmortem matrices such as femoral blood; vitreous humor and cerebrospinal fluid; with acetonitrile as an internal standard. Addition of an internal standard (acetonitrile) was ensured to authenticate results and compensate for any matrix changes. A good peak resolution between volatiles and internal standard was achieved. Linear correlation was achieved for peak area and concentration across the range of 3.95mg to 316mg/100ml with a correlation coefficient within the range of 0.988–0.999 for all matrixes. Limit of Quantitation (LOQ) and Limit of Detection (LOD) were within the range of 0.1mg%–1mg% for all matrixes. Reproducibility of samples and standards, inter- and intra-day, resulted in precision and accuracy within the range of acceptance as prescribed by validation guidelines. The sample volume required for validation was 1ml. With the developed method, no sample preparation or pre-treatment was required for volatile estimation. The total run time of the GC and HS cycle was 24.68min after sample sealing. The advantage of simultaneous screening of volatiles is important in forensic toxicology because of the possibility of identification of volatiles in single run, less sample utilized, and easy results for interpretation.

In conclusion, the proposed methodology serves to analyze the compounds, quantitatively and qualitatively utilizing minimum sample volume, and analysis of different biological fluids with the same method resulted in a methodology competent enough to be used in research and forensic samples.

#### **Postmortem Alcohols, Gas Chromatography, Validation**



### K32 Ethylenediamine Opioid Analogs, AH-7921, and U-47700 and Their Actions on Cloned Human OPRM1 Receptors

John L. Krstenansky, PhD\*, Keck Graduate Institute School of Pharmacy, Claremont, CA 91711; Alexander C. Zambon, PhD, Keck Graduate Institute, Claremont, CA 91711; Thomas Hsu, PhD, Keck Graduate Institute, Claremont, CA 91711; Jayapal Mallareddy, PhD, Keck Graduate Institute School of Pharmacy, Claremont, CA 91711

**Learning Overview:** After attending this presentation, attendees will learn about the development of an *in vitro* human mu-opioid receptor assay (hOPRM1) and the testing of analogs related to emerging synthetic opioids AH-7921 and U-47700.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing attendees to see how human receptor data compares to literature data that had only been performed in animals for some important emerging opioids of abuse.

The published pharmacology for the AH-7921 and U-47700 is more than 35 years old and was performed using non-human receptor systems.<sup>1-5</sup> While animal data and data using non-human receptors suggest similar action in humans, it is clearly more desirable to use human receptors to more accurately predict activity in humans. Considering the recent abuse of these opioids, this study sought to assess their pharmacology using a cloned human  $\mu$ -opioid receptor (hOPRM1) in an *in vitro* stable human cell line.

Analogs within the AH-7921 and U-47700 series were synthesized and characterized by Nuclear Magnetic Resonance (NMR), Gas Chromatography/Mass Spectrometry (GC/MS), Infrared (IR), and Raman (1,064nm and 785nm). In the U-4770 series, the stereochemically pure R,R and S,S isomers were synthesized from single isomer intermediates.

An amino terminal HA-tagged hOPRM1 receptor was stably expressed using lentiviral transduction in human fibrosarcoma HT1080 under control of the EF1a promoter. Agonists acting at the hOPRM1 receptor activate  $G_{ai}$ , which in turn suppresses cAMP levels. Therefore, to characterize hOPRM1 pharmacology, analogue-mediated suppression of forskolin-induced cAMP accumulation was measured. In addition, to determine if changes in cAMP were mediated by hOPRM1 receptors, a treatment group containing 1mM naloxone in addition to the highest dose of the test analogue was assessed. The levels of cAMP were measured using a commercial kit, ELISA-based Catchpoint assay. Optimization of the assay involved dose ranging the amount of naloxone needed to show complete agonist reversibility, buffer optimization to maintain cell adherence and minimize cell loss during the assay, and optimizing the sequence of reagent introduction to obtain consistent results.

The optimized assay was used to run full-concentration response curves for morphine, AH-7921, and U-47700 (R,R stereochemistry). The EC<sub>50</sub> values were 39nM, 16nM, and 10nM, respectively. These values are in agreement with the potencies reported in earlier literature. The (S,S) isomer of U-47700 had significantly less potency at this receptor. The literature suggests these analogs bind to both mu- and kappa-opioid receptors (OPRM1 and OPRK1) but with differing affinities based on their stereochemistry.<sup>3</sup> In a variety of series related to AH-7921 and U-47700, the 3,4-dichlorobenzoyl substitution consistently demonstrated the greatest potency relative to other substitutions on the benzoyl group.

In conclusion, an *in vitro* assay that can assess the human abuse potential of emerging opioids has been developed and the stereochemistry of the U-47700 stereoisomer more liable for abuse, (R,R), has been confirmed.

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#### Synthetic Opioids, AH-7921, U-47700



### **K33 The Extraction and Quantification of Cocaine and Its Metabolites From Fly Pupae**

*Shelby M. Sarginson\**, York College of Pennsylvania, York, PA 17403; *Kerry L. Opel, MA, PhD*, York College of Pennsylvania, York, PA 17403

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**THIS ABSTRACT WAS NOT PRESENTED.**



### **K34 An Analysis of Alcohol Metabolites Ethyl Glucuronide and Ethyl Sulfate in Human Umbilical Cord Samples**

*Marykathryn Tynon Moody, MSFS\*, NMS Labs, Willow Grove, PA 19090; Joseph Homan, MS, NMS Labs, Willow Grove, PA 19090; Denise Nicole Schiller, MSFS, Bristol, PA 19007; Wendy R. Adams, PhD, NMS Labs, Willow Grove, PA 19090; Frederick Strathmann, PhD, NMS Labs, Willow Grove, PA 19090; Barry K. Logan, PhD, NMS Labs/Center for Forensic Science Research and Education, Willow Grove, PA 19090; Robert A. Middleberg, PhD, NMS Labs, Willow Grove, PA 19090*

**Learning Overview:** After attending this presentation, attendees will be able to discuss a method for detecting ethyl glucuronide in human umbilical cord samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the capability of an analytical method that can be used to analyze ethyl glucuronide in umbilical cord samples as a biomarker of alcohol use during pregnancy.

Alcohol is the most popular legal psychoactive drug used in the United States. Due to its use and abuse across a variety of ages, races, and genders, the concern for prenatal exposure to ethanol is one to be taken seriously. While approximately 90% of pregnant women between the ages of 15 and 44 years old report they are abstaining from alcohol, maternal self-report is not always reliable. Many women do not want to self-incriminate or face the social stigma of reporting alcohol use; also many women reporting data may inadvertently have recall bias skewing the overall percentages. A more reliable and objective method to identify prenatal ethanol exposure would be beneficial to allow more timely intervention if needed.

The two alcohol metabolites focused on in this project are ethyl glucuronide and ethyl sulfate. These metabolites are formed by ethanol undergoing conjugation with either a glucuronide or a sulfate group, respectively. The detection of ethyl glucuronide has been reported in multiple newborn specimen types; however, there is very little data on ethyl sulfate.

This presentation describes a screening and confirmation method for the analysis of ethyl glucuronide and ethyl sulfate using a homogenizing-crash extraction with Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

The method was validated according to a Scientific Working Group for Forensic Toxicology (SWGTOX) -compliant procedure for a qualitative assay including experiments that evaluated precision around the decision concentration (cut-off), sensitivity and specificity, robustness, evaluation of interfering compounds, matrix effect, and extraction efficiency and stability of the analyte in extract and post-extraction stability.

Sample preparation for both the screening and confirmation methods consisted of an external cleaning process to remove contamination from the samples, followed by homogenizing the sample coupled with a protein precipitation. The extract was then diluted prior to instrumental analysis. The analytical method was performed on an AB SCIEX™ 6500+ triple quadrupole mass spectrometer equipped with an Electrospray Ionization (ESI) source for both methods. Separation was achieved using a Waters® CSH C18 (3mm x 100mm, 3.5micron) column coupled with 2 Phenomenex Security Guard Cartridges Polar-RP for the screening assay and a Waters® HSS T3 (3mm x 100mm, 3.5micron) column coupled with 2 Phenomenex Security Guard Cartridges Polar-RP for the confirmation assay.

These methods produced data that met the acceptance criteria established for the validation. Both methods produced 94% sensitivity and 100% specificity during the validation. It was also determined that there was no significant carry over after a sample of high concentration, and that there were no interferences when looking at 39 related compounds and common drugs of abuse. The analyte was stable in the extract for seven days, and stable in the auto-sampler vial for three days post-initial analysis. During development and validation, 138 patient cases were analyzed for ethyl glucuronide, and 124 cases for ethyl sulfate, and it was determined the estimated positivity rate for ethyl glucuronide was 4.35% and 100% for ethyl sulfate. Due to the 100% positivity rate for ethyl sulfate, it was removed from the scope while more information can be obtained.

#### **Ethyl Glucuronide, Umbilical Cord, Forensic Toxicology**



### K35 2,4-Dinitrophenol (2,4-DNP)—An “Old New Drug”: A Gas Chromatography/Mass Spectrometry (GC/MS) Method for Screening and Quantitation of 2,4-DNP in Postmortem Blood Specimens and Three Fatal Poisoning Cases Reports

Eduardo G. de Campos, MSc\*, Center for Forensic Science Research and Education, Willow Grove, PA; Melissa Friscia, MSFS, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Barry K. Logan, PhD, NMS Labs/Center for Forensic Science Research and Education, Willow Grove, PA 19090

**Learning Overview:** After attending this presentation, attendees will be able to describe a GC/MS method for the toxicological analysis of 2,4-DNP in blood and its concentration in authentic postmortem specimens.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel method for the analysis of 2,4-DNP in whole blood specimens and by reporting concentrations of 2,4-DNP found in authentic postmortem blood samples for future reference.

**Introduction:** 2,4-DNP is a substance that has been used as a pesticide and for manufacturing explosives and dyes. In 1933, 2,4-DNP was initially used as a weight-loss drug, but it was prohibited for human consumption in 1938 due to its serious adverse effects, including extreme loss of weight, hyperthermia, fatigue, respiratory depression, and death. In recent years, there have been reports of 2,4-DNP reemerging as a weight-loss drug and of associated lethal intoxication induced by this compound.

**Methods:** Sample preparation was performed by Liquid-Liquid Extraction (LLE). The samples were pretreated with a clean-up basic extraction using 0.1 N NH<sub>4</sub>OH, followed by an acidic extraction using 0.1 N HCl to target the 2,4-DNP, using n-butyl-chloride as the extraction solvent. 4-nitrophenol was used as the internal standard. Samples were rotated for 15min and centrifuged for 10min at 4,600rpm. After the final acidic extraction, the organic layer was collected and dried under nitrogen flow at 35°C for 30min. The extract was reconstituted in N,O-Bis(Trimethylsilyl)Trifluoroacetamide (BSTFA) -1% Trimethylchlorosilane (TMCS) and derivatized for 30min at 80°C. Analysis was performed by GC/MS, with electron impact ionization, and using hydrogen as the carrier gas. An HP-5MS column (30m x 0.2mm x 0.25um) was used and the total run time was 8.5min. Injector, MS source, and MS quadrupole temperatures were set to 265°C, 230°C, and 150°C, respectively.

**Results/Discussion:** A method with no derivatization was first evaluated using GC/MS with hydrogen and helium as carrier gases. However, underivatized-2,4-DNP was only detected at elevated concentrations using helium as the carrier gas. This study further evaluated a BSTFA derivatization step to increase the sensitivity. A recovery of 84% for 2,4-DNP was achieved with the LLE method. The method also showed good linearity in the range of 1.0mg/L–100mg/L (R<sup>2</sup>=0.9979).

Postmortem blood samples collected during autopsies from three suspected 2,4-DNP-related cases were analyzed. Case 1 was a 21-year-old male who was admitted to the emergency room after allegedly ingesting about 3mL of wintergreen oil. He presented with shortness of breath, increased body temperature, sweating, and increased heart rate. A family member stated that he had been consuming a thermogenic supplement, and a bottle of 2,4-DNP with an Aldrich® label was found at his house. The concentration of 2,4-DNP in his blood collected at the time of autopsy was 48mg/L. Case 2 was a male who presented with a 2,4-DNP concentration in postmortem iliac blood of 13mg/L. Case 3 was a female who was found dead and had a weight-loss supplement in her purse containing 2,4-DNP. A concentration of 3.3mg/L was found for 2,4-DNP in femoral blood.

**Conclusions:** 2,4-DNP is a highly polar phenolic compound and its detection by GC is an analytical challenge because 2,4-DNP shows a strong interaction with the stationary phase. In addition, 2,4-DNP derivatization by silylation with BSTFA seems to be very sensitive to reaction medium—better results were achieved when the derivatization was promoted with no solvent in the reaction medium (as acetonitrile). In this work, a GC/MS-based method, using hydrogen as carrier gas, for 2,4-DNP detection and quantitation was successfully developed, presenting a high potential to be easily implemented and performed in routine toxicological analysis. As the 2,4-DNP seems to be reemerging as a weight-loss drug, the method can detect and quantify 2,4-DNP in authentic human whole blood samples.

#### 2,4-Dinitrophenol, Gas Chromatography, Postmortem Toxicology





### K36 Quantitation of Fentanyl and Metabolites From *Lucilia Sericata* Larvae and Liver Tissue Using a Modified Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Extraction With Analysis by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Joseph A. Cox, MS\*, Pasadena, TX 77504; Colby Ott, BS, Shippensburg, PA; Luis E. Arroyo, PhD, Department of Forensic and Investigative Sciences, Morgantown, WV 26506-6121

**Learning Overview:** After attending this presentation, attendees will better understand a modified QuEChERS extraction combined with a sensitive method for determining concentrations for fentanyl and two known metabolites, norfentanyl, and despropionyl fentanyl (4-ANPP), in tissue toxicology specimens using LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by relaying a novel procedure that utilizes the unique extraction properties of a QuEChERS extraction with modifications to allow for the extraction of drugs from challenging matrices. Analytical separation and detection was conducted on a Triple Quad LC/MS/MS that provides the required sensitivity and selectivity of the target analytes.

The opioid crisis in the United States has a serious impact on our society. According to the National Institute on Drug Abuse (NIDA), more than 100 people die every day of opioid overdose. Even more concerning is the increase of overdose deaths involving synthetic opioids (e.g., fentanyl and fentanyl analogs), with increases from 3,105 deaths in 2013 to 19,413 deaths in 2016 (625% increase). Under special circumstances (e.g., when blood or urine are not available due to decomposition or exsanguination), liver samples are regularly the first choice for toxicological analysis, mainly due to the organ's ease of collection at autopsy and homogenization for drug extraction. On the other hand, fly larvae have been used as a toxicological analysis matrix when traditional matrices are not available due to skeletonization or to corroborate findings in extremely decomposed tissues. The succession that occurs on a corpse after death is a relatively confined and closely packed ecosystem that is typically restricted to the remains and close proximity. If xenobiotics are present in the body, they will be ingested by the organisms feeding on the corpse. Therefore, any concentrations identified in insect tissue can be indicative of drugs present in the tissue they were feeding on.

Common practices for tissue extraction involve a Solid Phase Extraction (SPE) or Liquid-Liquid Extraction (LLE), but the process for these techniques can be time consuming and often involve tissue homogenizers using blenders that could allow cross contamination. In recent years, the QuEChERS extraction protocol was introduced in the market to deal with samples with high a content of fatty materials, especially for food analysis. This sample preparation technique avoids the risk of cross contamination by producing a homogenized sample into a single disposable tube. QuEChERS has been reported as a rugged sample preparation method in blood analysis and more recently in liver tissues. This study proposed using a simple sample homogenization system consisting of a high-speed mixer mill with disposable stainless-steel balls contained in a homemade plastic holder. This setup helped to reduce the sample size to a few grams and improve the homogenization steps. A modified QuEChERS extraction was used with a 0.2g sample (liver and larvae) amount. Homogenization was achieved using a high-speed mixer mill (Retsch MM 200) with a homemade attachment for 1.5mL centrifuge tubes and 4.5mm stainless steel balls. The quantification method was performed on an Agilent® 6470 Triple Quad LC/MS/MS system. Chromatographic separation was achieved on a ZORBAX® Eclipse Plus™ C18 RRHD 3.0mm x 100mm, 1.8µm column with 0.1% formic acid and 5mM ammonium formate in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B). *Lucilia sericata* eggs (250) were placed to feed on beef liver tissue homogenate (200g) with four concentrations of fentanyl (0µg/Kg, 2. µg/Kg, 50µg/Kg, 250µg/Kg) and allowed to develop to third instars. Thirty specimens from each treatment were heat killed in water, rinsed to remove any potential residual drug, then dried, measured (weight, length, and width) and stored at -80°C for toxicology analysis.

A validation of the LC/MS/MS method for fentanyl, norfentanyl, and 4-ANPP was performed with a Limit Of Detection (LOD) of 0.1ppb and an Analytical Measurement Range (AMR) of 0.5ppb to 250ppb. Accuracy for all three compounds at the lowest calibrator were above 82 (±0.88) % with a %RSD of less than 5% and for the highest calibrator above 94 (±0.47) % accuracy with a %RSD less than 0.5%. Linearity was acceptable for all three compounds as they were all better than 0.995. The average recovery for the liver extraction was greater than 75 (±1.4) % and the average recovery for the larvae extraction was greater than 71 (± 1.6) %.

The method was validated, and the calibration curves reconcile well with forensic toxicology criteria. The extraction and LC/MS/MS method developed for analysis of larvae and liver tissue for fentanyl, norfentanyl, and 4-ANPP is precise, sensitive, and reproducible at forensically relevant concentrations.

#### QuEChERS, Fentanyl, *Lucilia sericata*

## K37 Study of the Mu-, Kappa-, and Delta-Opioid Receptor Models and the Kratom Alkaloids

Reba E. Chamblee\*, Fulton, MS 38843; Caroline Spencer, BS, Oxford, MS 38655; Pankaj Pandey, PhD, University of Mississippi, University, MS 38677; Robert J. Doerksen, PhD, University of Mississippi, University, MS 38677; Murrell Godfrey, PhD, University of Mississippi, University, MS 38677

**Learning Overview:** After attending this presentation, attendees will better understand the interactions of kratom and its various alkaloids with the mu-, kappa-, and delta-opioid receptors.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the understanding of complex interactions between substituents of specific kratom alkaloids with the mu-, kappa-, and delta-opioid receptors. Understanding these key interactions can help lead to improved knowledge of the effects these alkaloids produce.

Kratom, *Mitragyna speciosa*, is a plant indigenous to countries of southeast Asia and has traditionally been used for medicinal treatments.<sup>1</sup> However, the popularity of the drug has significantly increased recently due to its euphoric effects, leading it to being used as an alternative to illegal opioids. Several alkaloid compounds have been isolated from the leaves of the plant. The main alkaloids seen are the following five alkaloids: mitragynine, 7-hydroxymitragynine, speciociliatine, speciogynine, and paynantheine. Two alkaloids, mitragynine and 7-hydroxymitragynine, have exhibited high potencies and are potentially even more potent than morphine.<sup>2</sup> Previous studies have indicated that the main mediator of the psychoactive effects is the opioid receptor system, specifically the mu-, kappa, and delta-opioid receptors.<sup>3</sup> Agonistic activity has been seen by mitragynine and 7-hydroxymitragynine at the mu-opioid receptor while antagonistic activity has been seen by all five of the above alkaloids at the kappa- and delta-opioid receptors. The highest binding affinities occur at the mu-opioid receptor and lesser affinities at the kappa- and delta-opioid receptors. Expanding concern of kratom has led to more research into its mechanism of binding with target receptors and how its growth of use impacts the forensic science and criminal justice community.

In this study, mu-, kappa-, and delta-opioid receptor models were used to establish the ligand-receptor interactions between the receptors and the constituents of specific kratom alkaloids. This was accomplished using Schrodinger's Maestro molecular modeling software. The major natural alkaloids of the plant were selected for this study because of their high abundances as well as their binding affinities. Maestro employs the technique known as molecular docking to study the binding of a ligand to the active site of a known 3D protein model. The docking of the ligands to proteins is used to determine the most accurate orientation of the ligand in the active site and the specific interactions that take place between the ligand and the protein. Maestro determines a "docking score" that is not the same as the experimental binding affinity but is related in terms of ranking the binding of the ligands to the receptor. Docking of the ligands to the receptors took place after both the alkaloid ligands and the opioid receptor models were prepped for docking and a grid of the active sites was generated. Data generated from each docking yielded information on the best poses/positions of the alkaloids for binding to the receptors, interactions between the ligands and receptors, and the estimated binding affinities.

The results of this study provide a better understanding of the interactions that take place between kratom alkaloids and the opioid receptors. Specific structural characteristics of the alkaloids studied were found to influence key residue interactions with the receptors. This study looked at structural characteristics, such as: length of the alkyl chain; presence or absence of function groups, including ketone, ester, and hydroxyl; and amine groups. Some residues were present consistently throughout the studies, including Asp147, Tyr148, and His297 in the mu-opioid receptor, Tyr129 in the delta opioid receptor, and Asp138 in the kappa opioid receptor. All these residues have been found to be important residues in activation of the opioid receptors.<sup>4</sup> The next step of this research will include comparing the docking studies with the different receptors to the known binding affinities to gain a better understanding of what is influencing the binding affinities and the adverse reactions of the kratom alkaloids. The results from this study will aid in the understanding of the influence specific structural characteristics have on the binding and pharmacology of these drugs. Future results from this study will lead to a new understanding of the effects kratom and its alkaloids have within the human body and help determine its potential for abuse.

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### Kratom, Opioid Receptors, Molecular Modeling



### **K38 Electrochemical Tools for the Rapid Detection of Opioids and Novel Psychoactive Substances (NPS) With Confirmatory Analysis by Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS)**

*Colby Ott, BS\*, Shippensburg, PA; Joseph A. Cox, MS, Pasadena, TX 77504; Hugo Cunha-Silva, MS, University of Burgos, Burgos, SPAIN; Julia Arcos-Martínez, PhD, University of Burgos, Burgos, SPAIN; Luis E. Arroyo, PhD, Department of Forensic and Investigative Sciences, Morgantown, WV 26506-6121*

**Learning Overview:** After attending this presentation, attendees will be able to explain the theory and use of electrochemical techniques as screening tools in forensic science and understand their implications in the testing of NPS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by advancing toward an electrochemical sensing method that will provide qualitative and quantitative information to enhance workflow in laboratories to speed up screening and analysis, reducing costs and backlogs. The proposed approach will allow on-site testing of unknown substances enabling for non-invasive roadside testing of Driving Under the Influence of Drugs (DUID) suspects and as a rapid on-site detection system on drug seizures.

Due to the growing number of cases involving fentanyl and fentanyl-analogs, opioid abuse poses a significant threat to the United States. Opioid-related overdose deaths have increased over the past several years, leading to a public health emergency declared by the Department of Health and Human Services. NPS have compounded the issue due to having similar or increased potency. NPS are synthetic analogs to known controlled substances designed by making modifications to the core chemical structure in most cases, and include fentanyl-analogs and synthetic cannabinoids, among others. Electrochemistry can provide a rapid, sensitive, and selective screening technique to overcome the limitations faced by other methods in the field. Electrochemistry offers a versatile platform that is sensitive, portable, and low cost, which can be modified to suit a variety of needs and detection requirements.

Cytochrome P450 enzymes represent a class of enzymes suited to work with controlled substances due to their role in the metabolism of the many xenobiotics within the body. Modification relies on the ability to transfer electrons from the working electrode to the heme center of the enzyme to stimulate the enzyme's metabolism of the drug in solution. Additional modification with gold nanoparticles provides a good metal platform that can be used for attachment strategies not possible on carbon, as well as a different electrode surface. Such modification is demonstrated in this study.

Screen-Printed Electrodes (SPEs) allow for a portable and straightforward testing platform due to containing all three needed electrodes within a small area. SPEs are made by depositing alternating layers of conductive material for the electrode surfaces with insulating material and plastic to create a small (~1.5 x 0.5 inches) strip-like testing surface. SPEs are desirable testing platforms due to being low cost and disposable, having high-throughput, and requiring small sample volumes. These SPEs then act as sensors, recording the electrochemical process taking place and can provide qualitative and quantitative data about an analyte in solution. The most common of these SPEs are carbon-based, which provide a large window of potential and possible modifications. These electrodes can be modified with many materials, including enzymes and nanoparticles/nanomaterials. Modifications allow for the activity, sensitivity, and specificity to be controlled for the desired application. Traditional electrochemical testing methodologies also allow for modifications to the electrode surface as well as various types of electrode materials.

Three target drugs were tested: codeine, fentanyl, and PB-22 (a synthetic cannabinoid NPS). Cytochrome P450 enzymes were immobilized on the surface of the electrodes for analysis of codeine. Cyclic voltammetry and chronoamperometry were utilized for the analysis and characterization of the drug in Phosphate-Buffered Saline (PBS). Standard curves from successive additions were generated to determine potential limits of detection. Analysis of fentanyl in PBS via deposited gold nanoparticles on the working electrode was tested to determine its viability as a testing method. Finally, a traditional electrochemical cell approach was utilized with a platinum working electrode for the analysis of PB-22 in acetonitrile containing tetrabutylammonium perchlorate. Additionally, a method using dynamic multiple reaction monitoring with LC/QqQ/MS was developed for confirmatory analysis as part of the development of a pain-management panel containing 28 drugs. This method was designed for use in oral fluid.

Codeine was characterized in PBS buffer, and the limit of detection was found in the low  $\mu\text{g/mL}$  levels. Similar ranges of detection were obtained for fentanyl. Detection of fentanyl was possible utilizing only gold nanoparticles on the SPE. Excellent linearity ( $>0.999$ ) was seen between  $3\mu\text{g/mL}$  and  $1\mu\text{g/mL}$  concentrations tested. The limit of detection of PB-22 was determined to be approximately  $208\text{ng/mL}$  with linearity greater than  $0.999$ .

#### **Electrochemistry, NPS, Opioids**



### K39 A Comprehensive Analysis of Synthetic Cannabinoids and Metabolites in Oral Fluid (OF) By Online Solid Phase Extraction (SPE) and Liquid Chromatography/Triple Quadruple/Mass Spectrometry (LC/QqQ/MS)

Carmen T. Mulet, BS\*, Florida International University, Miami, FL 33199; Anamary Tarifa, PhD, Florida International University, Miami, FL 33199; Anthony P. DeCaprio, PhD, Florida International University, Miami, FL 33199

**Learning Overview:** The goal of this presentation is to demonstrate the comprehensive analysis of 30 synthetic cannabinoids and metabolites in OF by LC/QqQ/MS using online SPE for sample pretreatment.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how to detect and quantify Synthetic Cannabinoid (SC) parent drugs, as well as metabolites, in a non-invasive matrix using a relatively swift analytical technique that is sensitive enough to be able to detect low levels of structurally diverse SCs.

According to the National Institute on Drug Abuse, the emerging class of novel psychoactive substances known as SCs include man-made, mind-altering chemicals that are sprayed on dry, shredded plant material and can either be smoked or sold as liquids to be vaporized in e-cigarettes. SCs are often marketed as “synthetic marijuana” and may not be detected by traditional drug screening tests for marijuana, as they do not have the same structure as  $\Delta^9$ -THC, the main active ingredient in marijuana. Due to the typically rapid metabolism of SCs and their instability in biological matrices, fast analytical methods are needed for the detection and confirmation of parent drugs, as well as metabolites, at very low levels.

SCs are transferred from the blood into OF primarily by diffusion. The detection of these drugs in an OF matrix is advantageous because there is no need for same-sex collection, it is a non-invasive technique that has little risk of adulteration, and collection can be done onsite. OF is a viscous aqueous matrix composed of electrolytes, small molecules, and higher molecular-weight compounds, including proteins and enzymes. Therefore, a sample pretreatment step, typically Liquid-Liquid Extraction (LLE) or SPE, is required prior to sample analysis. Unfortunately, both procedures are time-consuming and involve large amounts of solvents for cleanup.

This study demonstrates the comprehensive analysis of 30 synthetic cannabinoids and metabolites in OF by LC/QqQ/MS using online SPE for sample pretreatment. The aim is to be able to detect and quantify SC parent drugs, as well as metabolites, in a non-invasive matrix using a relatively swift analytical technique that is sensitive enough to be able to detect low levels of structurally diverse SCs. OF specimens are collected using a Quantisal<sup>®</sup> device consisting of a cotton pad that is placed in the mouth and turns blue upon collection of an approximate 1mL OF sample. This is transferred to a plastic collection tube containing 3mL buffer, provided by the manufacturer, for drug extraction and stability during storage. An aliquot from this sample is spiked with an internal standard SC mix and compared against a six-point calibration curve created using synthetic or authentic pooled OF and buffer (1:3, v:v). An Agilent<sup>®</sup> 6470 triple quadrupole MS equipped with Jet Stream Electrospray Ionization (ESI) technology is used in positive mode with dynamic Multiple Reaction Monitoring (MRM) (3 MRM repeats) for the characterization of the analytes (1 $\mu$ L injection). Binary gradient elution is performed on a ZORBAX<sup>®</sup> Eclipse Plus<sup>™</sup> C18 Rapid Resolution HD (3.0 x 100mm x 1.8 $\mu$ m) column using MeOH + 0.1% formic acid as organic phase and 5mM ammonium formate + 0.1% formic acid as phase modifier with a total run time of 15min. Online SPE is carried out on an Agilent<sup>®</sup> 1290 Infinity<sup>®</sup> Flexcube using ACN, MeOH, and water for cleanup and reconditioning.

Initial results demonstrate calibration curve linearity from 0ng/mL–100ng/mL ( $R^2 >0.99$ ), with Limit Of Detections (LODs) in the range of 0.5ng/mL–1ng/mL. In addition, better analyte ionization was noted with authentic pooled OF as compared to synthetic OF for calibration curves. Determination of Matrix Effects (ME), Process Efficiency (PE) and Recovery (RE) showed data for most analytes to be within acceptable limits according to the Scientific Working Group for Forensic Toxicology (SWGTOX) and other relevant validation guidelines. An exception was noted in the case of PB-22 metabolites, which showed ME >198%, PE >283% and RE >135%. Following full validation and inclusion of additional SCs and metabolites, this method is expected to facilitate rapid and reliable screening of SCs in forensic toxicology applications.

#### Synthetic Cannabinoids, Oral Fluid, Online SPE



### K40 Investigating the Binding of Fentanyl and Fentanyl Analogs to the Opioid Receptors

Marissa A. Teske\*, Olive Branch, MS 38654; Caroline Spencer, BS, Oxford, MS 38655; Pankaj Pandey, PhD, University of Mississippi, University, MS 38677; Robert J. Doerksen, PhD, University of Mississippi, University, MS 38677; Murrell Godfrey, PhD, University of Mississippi, University, MS 38677

**Learning Overview:** After attending this presentation, attendees will better understand the interactions between fentanyl and its various analogs with the mu-, kappa-, and sigma-opioid receptors in the brain.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the understanding of the interactions between fentanyl, fentanyl analogs and the mu-, kappa-, and sigma-opioid receptors, and of the specific interactions that are related to the adverse effects of these drugs.

Fentanyl was synthesized in 1960 by Dr. Paul Janssen of the Janssen Company to act as a rapid-acting analgesic that, unlike similar analgesics around that time, did not have negative cardiovascular effects.<sup>1</sup> Fentanyl overdoses were first reported around 1972 with subsequent increases in overdose cases reported as additional methods of administration became available.<sup>1</sup> From 1999 to 2011, the number of fatal opioid analgesic overdoses quadrupled and, for the year 2015, the death toll in the United States totaled 33,091.<sup>2</sup> The lacing of heroine with fentanyl by drug dealers in order to increase the client's opioid high is one major reason for this spike in overdose deaths. Fentanyl and its analogs are all agonists of the opioid receptors.<sup>2</sup> There are several physical effects of opioid binding at the  $\mu$ -opioid receptor, including respiratory and central nervous system depression.<sup>2</sup> Fentanyl-related deaths are increasing rapidly, and more potent fentanyl analogs are found laced in heroine and cocaine every day. It is therefore imperative that scientists stay ahead of the illegal drug trade. One way this goal can be accomplished is by using cutting-edge technology to investigate ligand binding and molecular docking of illegal substances before they even enter the market.

This study examined the interactions that take place between fentanyl, and its analogs, and the opioid receptors they interact with in the body. The molecular modeling software, Maestro, was used to study these specific interactions. Fentanyl and its analogs were sketched and prepped for docking to the receptors using Maestro. The opioid receptors were chosen for this study from available, active-state crystal structures. The data from this study identified specific interactions that take place between these drugs and the binding site of corresponding opioid receptors. Key residue interactions included aromatic stacking interactions, hydrophobic interactions, polar interactions, and hydrogen bonding. These interactions corresponded to varying structural changes between fentanyl and its analogs. The interactions and structural changes were used to help better understand the potency and toxicity of fentanyl and fentanyl analogs. Future work will include using the results of this study to help predict potential new analogs of fentanyl before they appear on the drug market. Scientists and law enforcement will have advanced knowledge on various fentanyl-related substances to improve both detection and treatment of fatal overdose cases.

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#### Fentanyl, Opioid Receptor, Molecular Modeling



**K41 WITHDRAWN**



## K42 Serum Albumin Binds Synthetic Cannabinoids and Model Compounds and Drugs

Oluseyi A. Vanderpuye, PhD\*, Albany State University, Forensic Science, Albany, GA 31705; Alexius K. Lampkin, Headland, AL 36345; Johnathan Jones, BS, Albany State University, Forensic Science, Albany, GA 31705

**Learning Overview:** After attending this presentation, attendees will learn: (1) how agarose gel electrophoresis and spectroscopy can be applied to detect binding of certain synthetic cannabinoids, prescription drugs, and compounds with serum albumin; and (2) that interactions of synthetic cannabinoids and drugs with serum albumin are a consideration for the pharmacokinetics of synthetic cannabinoids and other compounds.<sup>1</sup>

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how serum proteins affect drug bioavailability and methods of characterizing drug-serum protein interactions. Additionally, the community will become more aware of the use of spectroscopic methods for characterizing interactions of different compounds with albumin. Interpretation of toxicological data may be influenced by agents that affect the blood concentration of free drugs and compounds and their lifetimes.<sup>2,3</sup> Albumin has demonstrated relevance for these parameters for many compounds of toxicological interest; therefore, consideration of its effects on pharmacokinetics of synthetic cannabinoids warrants more investigation.

Serum albumin is known to bind a diversity of non-polar organic compounds and drugs.<sup>1-4</sup> It was hypothesized that human and bovine serum albumin would bind to synthetic cannabinoids such as Bay 59-3074 and JWH-015, which are non-polar molecules with ring structures that are practically insoluble in aqueous solution.

For this study, binding to albumin was tested for the synthetic cannabinoids and as controls, model compounds such as the dye Nile Red, eosin, bromophenol blue, coumarin, the drugs tobramycin, colchicine, and a brand name mixture of brimonidine tartrate and dexamethasone to further advance knowledge of compounds that bind serum albumin.

Agarose gel electrophoresis was one method used for assessing binding of the synthetic cannabinoids and other compounds to albumin. Ultraviolet/Visible (UV-Vis) and fluorescence spectroscopy were other applicable methods.<sup>3,4</sup> The compounds used can be visualized by their color or by their UV-induced fluorescence. In the absence of BSA, non-polar synthetic cannabinoids and model compounds would not be expected to migrate in agarose gels or be visible after electrophoresis. If any of these compounds bind to albumin, then their complexes would migrate in agarose gel electrophoresis bound to the albumin and be visible by their intrinsic color or fluorescence under UV-light.

It was found that in agarose gel electrophoresis in the presence but not in the absence of albumin, fluorescent bands at the position of albumin were observed for mixtures with Bay 59-3074 and JWH-015 and for mixtures of albumin with Nile Red, eosin, coumarin, the drugs tobramycin, colchicine and mixture of brimonidine tartrate and dexamethasone. In the absence of these compounds, albumin was not fluorescent or colored. It was also observed that in the presence of albumin, compounds such as eosin, bromophenol blue and Nile Red changed color. In the presence of albumin, under UV-light, Bay 59-3074, JWH-015 and Nile Red became more fluorescent. This suggested that interaction of these and other compounds would be detectable by changes in the UV-Vis and fluorescence spectra.

In conclusion, it was observed for the first time that the synthetic cannabinoids Bay 59-3074 and JWH-015 bind serum albumin. This could affect their bioavailability and allow these drugs to attain higher concentrations in blood than permitted by their non-polar character and allow them to circulate longer. The use of agarose gel electrophoresis to detect albumin binding to synthetic cannabinoids and other compounds has not been previously reported and is applicable to the study of albumin interaction with compounds of pharmacological significance that are fluorescent or colored.

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### Cannabinoids, Albumin, Electrophoresis





## K43 Postmortem Distribution of Nicotine and Cotinine in Suicidal Overdoses Following Transdermal and Oral Administration

Rebecca Jufer Phipps, PhD\*, State of MD, Office of the Chief Medical Examiner, Baltimore, MD 21223; Saffia Ahmed Sakinedzad, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223; Mary G. Ripple, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223; Ling Li, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223; David R. Fowler, MD, Office of the Chief Medical Examiner, Baltimore, MD 21223

**Learning Overview:** After attending this presentation, attendees will have a better understanding of the postmortem distribution of nicotine and cotinine following transdermal and oral administration.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing medical examiners and toxicologists with postmortem concentrations for nicotine and cotinine in suicidal overdoses following transdermal and oral administration.

Nicotine is a toxic alkaloid present in tobacco at concentrations (w/w) of 0.5%–8%. In addition to cigarettes and smokeless tobacco products, nicotine is commercially available in lozenges, sublingual tablets, chewing gum, nasal sprays, inhalers, transdermal patches, and solutions for electronic smoking devices. Nicotine produces stimulation of nicotinic receptors in the autonomic ganglia and central nervous system. Nicotine overdose produces symptoms ranging from nausea, dizziness, tachycardia, and hypertension to convulsions, coma, and death. Blood nicotine concentrations ranging from 1.0mg/L to >100mg/L have been reported in nicotine associated fatalities.<sup>1</sup>

The Office of the Chief Medical Examiner (OCME) of the State of Maryland recently investigated two deaths due to nicotine intoxication; one involving transdermal administration and the other involving oral ingestion of a vape liquid.

**Case 1 History:** A 60-year-old female was found unresponsive in her residence during a welfare check (requested by friends who had received suicide letters mailed by the decedent). She was lying in a supine position on her bed with a suicide note located on the bedside table. Two empty, unlabeled pill bottles and a taped recording of the decedent were also located in the bedroom. Due to case history, an inspection at OCME was performed. External examination revealed 14 21mg/24hr nicotine patches on her body (ten on her chest, one on each arm and leg). Other than the nicotine patches, inspection findings were unremarkable. Comprehensive toxicology testing was performed, and the results are summarized in the table below.

**Case 2 History:** A 34-year-old female was found unresponsive in the driver's seat of a vehicle parked in a residential neighborhood. She had been reported missing two days earlier by her mother who reported that the decedent indicated that she was going to kill herself by "poisoning." Five empty bottles of Big Heart 555 vape liquid (12mg/34mL) and an empty water bottle were present in the vehicle. A full autopsy was performed, and findings were unremarkable. Comprehensive toxicology testing was performed, and the results are summarized in the table below.

Comprehensive toxicology testing included volatiles, an acidic neutral drug screen, an alkaline drug screen and Enzyme-Linked Immuno-Sorbent Assay (ELISA) for morphine, benzodiazepines, and oxycodone. Nicotine was confirmed and quantitated by liquid-liquid extraction, followed by Gas Chromatography/Mass Spectrometry (GC/MS). Briefly, internal standard (d<sub>4</sub>-Nicotine; 0.2 mg/L) was added to specimens (5.0mL) that were alkalized with 0.1N sodium hydroxide and extracted with n-butyl chloride, then back extracted into 0.1N sulfuric acid, and finally alkalized with ammonium hydroxide and extracted into methylene chloride. Isopropanol (100µL) was added and the extract was evaporated to the isopropanol layer which was injected into the GC/MS for analysis. The method was linear from 0.12mg/L to 2.0mg/L for nicotine and cotinine. A five-point calibration curve and two control concentrations (0.30mg/L and 1.25mg/L) were used for quantitation. An administrative reporting limit of 0.12mg/L for both analytes was used. Toxicology results (mg/L or mg/kg) for the two cases are summarized below.

Case	Heart Blood	Peripheral Blood	Liver	Kidney	Vitreous Humor
1	Nicotine: 0.20	Nicotine: 0.22	N/A	N/A	Nicotine: 0.12
	Cotinine: 0.36	Cotinine: 0.48			Cotinine: 0.32
	Alprazolam: 0.033				
	Dextromethorphan: 0.5				
	Diphenhydramine: 0.6				
	Fluoxetine: 1.1				
	Hydroxyzine: 0.4				
Oxycodone: 1.2					
2	Nicotine: 34	Nicotine: 21	Nicotine: 170	Nicotine: 31	Nicotine: 9.9
	Cotinine: 0.40	Cotinine: 0.30	Cotinine: 1.2	Cotinine: 0.35	Cotinine: <0.6
	Diphenhydramine: 0.05				

For Case 1, the medical examiner ruled that the cause of death was nicotine, oxycodone, fluoxetine, and dextromethorphan intoxication; the manner of death was suicide. For Case 2, the medical examiner ruled that the cause of death was nicotine intoxication; the manner of death was suicide. The nicotine concentrations detected in these two cases are significantly elevated from what would be expected with appropriate use of the products involved. In Case 1, it is likely that higher nicotine concentrations were not observed due to the presence of additional drugs at toxic concentrations and the slower transdermal route of administration. In Case 2, the nicotine concentration was very high with lower concentrations of cotinine, suggesting that death occurred quickly after administration of an excessive dose.

### Reference(s):

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### Nicotine, Postmortem, Overdose



### K44 Surface Enhanced Raman Scattering (SERS) Analysis for Synthetic Opioids

Xuyang He\*, University of Southern Mississippi, Hattiesburg, MS 39406

**Learning Overview:** After attending this presentation, attendees will understand: (1) how sensitive and useful the SERS technique is in forensic drug/toxicological analysis, (2) how to optimize the signal enhancement to various degrees by using different metal substrate/solvent/aggregating reagents, and (3) how to do a quantitative measurement with SERS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by elucidating the application of SERS in forensic investigations regarding synthetic opioids. In most cases, SERS was deemed a rapid and sensitive screening test, but SERS can also work as a reliable confirmatory test if used properly.

Presented here is the study of identifying and quantifying certain synthetic opioids using SERS. In recent years, the abuse of opioids, especially the powerful synthetic opioids such as fentanyl and its analogs, has become America's leading cause of overdose deaths, which is the so-called opioids crisis. Hence the rapid detection, identification, and quantification of these psychoactive substances emerge as a critical need for forensic scientists. Usually it is not feasible to analyze fentanyl and its analogs using traditional techniques because of their superior potency, leading to very low concentration present in samples. As a valuable analytic tool for the detection and identification of various chemical compounds of forensic interest, Raman spectroscopy attracts increasing attention over the past decades due to its non-destructive nature, wide application range, and capability of providing rich structural information. The Achilles' heel of general Raman spectroscopy is its weak signal, resulting from the fact that only one scattered photon out of ten thousand is Raman active. One of the solutions to this is the SERS technique, which boosts the signal by placing the sample in the vicinity of or on rough metal/semiconductor substrates. The signal enhancement factor of SERS can reach up to  $10^{11}$ , improving the detection limit to  $10^{-12}$  M. In addition, SERS has proven to be a great means to quench unwanted luminescence for fluorescent molecules while enhancing the Raman signal at the same time.

This study analyzed synthetic opioids, especially those potent ones such as ohmefentanyl, tetrahydrofuranlylfentanyl, etc. Multiple research efforts have been focused on qualitative detection and identification of forensic drugs by SERS. However, quantitative SERS measurements are scarce, owing to the complexity of the system and the spectra. Ideally, when SERS peaks of interest are unique and not overlapped with other peaks, quantification is possible by calculating peak areas/heights after appropriate calibration. In fact, an analyte is always within a complex background such as blood/urine/saliva plus metallic colloidal solutions and we often need to perform quantitation for multiple analytes simultaneously. One way to overcome these issues is the Standard Addition Method (SAM), by spiking the standard to both blank and real samples. Therefore, first this work systematically studied the dependence of SERS signal enhancement on difference experiment conditions. The results reflect how the signal enhancement varied with different substrates, solvents, and aggregating reagents. Also, the quantitative measurements of certain potent synthetic opioids under optimized SERS conditions are conducted using the standard addition method.

#### SERS, Opioids, Quantitation



**K45 WITHDRAWN**



### **K46 The Interplay Between Forensic Analysis of Seized Drug and Medicolegal Death Investigation Toxicology in the Investigation of Clinical Intoxications to Support Public Health Preparedness and Response**

*Barry K. Logan, PhD\*, NMS Labs/Center for Forensic Science Research & Education, 3701 Welsh Road, Willow Grove, PA 19090; Alex J. Krotulski, MS, Center for Forensic Science Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090; Amanda L.A. Mohr, MSFS, Center for Forensic Science Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090*

**Learning Overview:** After attending this presentation, attendees will be able to evaluate the benefits of an integrated approach to early identification of toxic Novel Psychoactive Substances (NPS) in forensic toxicology and seized drug casework and the rapid communication of these findings.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing the interconnectedness of forensic analysis of seized drug and medicolegal death investigation casework, with investigation of clinical intoxications in support of public health functions.

There are increasing demands from public health and public safety agencies for better data regarding the extent, trends, and impacts of adverse reactions to the current opioids being abused. Comprehensive chemical analysis, market awareness to inform scope of testing, data collection and management, and rapid sharing of information are all key components of ensuring that both clinical and forensic practitioners remain aware of the most current threats from novel opioids. Data from drug identifications made in seized drugs directs the generation and updating of mass spectral libraries for both point of contact screening devices, seized drug laboratories, and toxicology screening protocols. Confirmation of positive findings from toxicological testing in clinical and forensic populations can alert seized drug testing labs of the need to perform additional testing.

This study has envisioned a model of using data from seized drug testing obtained from samples at ports of entry into the United States, with data from the sample-mining and data-mining of toxicological samples from medicolegal death investigation cases, and data from comprehensive toxicological testing of intoxications in the emergency room, to rapidly identify threats to public health and prepare the relevant stakeholder groups to respond.

In July 2018, there was a mass outbreak of apparent opioid overdoses in Philadelphia, PA, on Saturday and Sunday, with more than 1,000 cases being reported. This was associated with an opioid stamp design “Santa Muerte.” Three deaths were attributed to the outbreak by the media. The cases were unusual in that once the subjects were reversed with naloxone, they displayed an uncharacteristic agitation, shaking, and tachycardia, which apparently responded to treatment with physostigmine, an antidote to anticholinergics such as scopolamine or atropine. Consequently, health alerts were sent out through the public health network about this threat from a combined opioid with anticholinergic.

The following Tuesday, a sample of the Santa Muerte product was submitted to the laboratory for testing. Analysis was performed the same day by Liquid Chromatography/quadrupole Time Of Flight/Mass Spectrometry (LC/qTOF/MS), and Gas Chromatography/Mass Spectrometry (GC/MS) using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) developed by the National Institute of Standards and Technology (NIST). The material was found to contain heroin, fentanyl, and 5F-ADB, a potent cannabinoid CB1 receptor agonist. No anticholinergic drug was present. 5F-ADB can co-elute with heroin under some chromatographic conditions and use of AMDIS can be key to identifying these minor components in complex drug mixtures.

On that Wednesday, samples from one of the intoxications was retrieved and was submitted to the laboratory for testing. Those samples were tested the following day and confirmed the presence of 6-monacetylmorphine, fentanyl, and 5F-ADB. Those results were returned to the hospital, and the Philadelphia Public Health Department was notified of the findings. The findings were also communicated to the National Drug Early Warning System (NDEWS), the Drug Enforcement Administration (DEA) SYNTH-OPIOIDS list, and a pre-established list of other key national and international stakeholders.

This early alert enabled public health agencies to share this information about the toxicity of this batch of drugs with the drug-using community to assist in harm reduction and reduce the risk of further adverse events, and it allowed local crime laboratories to re-examine their chromatographic data and identify 5F-ADB in prior cases. It alerted toxicology laboratories to consider including synthetic cannabinoid testing in their investigation of apparent drug injection deaths or intoxications. It also raised awareness among medical toxicologists of the potential for synthetic cannabinoid drugs to manifest as anticholinergic intoxications, improving the potential for future treatments.

This case study showcases the importance of the interplay between toxicology and seized drug analysis, clinical and forensic stakeholders, and public health and public safety organizations in the investigation of NPS intoxications. It also reinforces the critical need for rapid and timely testing in emergent outbreaks to maximize the value of this resource.

#### **Synthetic Cannabinoids, Novel Opioids, Early Warning Systems**

## K47 The Identification of Synthetic Cannabinoids in Forensic Toxicology Casework Using an Archived High Resolution Mass Spectrometry Data System

Amanda L.A. Mohr, MSFS\*, Center for Forensic Science Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090; Alex J. Krotulski, MS, Center for Forensic Science Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090; Barry K. Logan, PhD, NMS Labs/Center for Forensic Science Research & Education, 3701 Welsh Road, Willow Grove, PA 19090

**Learning Overview:** After attending this presentation, attendees will be able to describe a rapid Liquid Chromatography (LC) high resolution Time-Of-Flight Mass Spectrometry (TOF/MS) method for the analysis of synthetic cannabinoids and workflow for the retrospective datamining of previously acquired sample extracts and TOF data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel approach for creating a historical data archive for biological specimens that will be continually re-interrogated as new synthetic cannabinoids and their metabolites are discovered to determine retrospectively the date of first appearance and spread of new compounds as they appear on the drug market.

Since 2008, synthetic cannabinoids have continued to infiltrate the drug market and have been implicated in an increasing number of emergency room admissions, death investigations, and high-profile intoxication events in corrections populations. Understanding the scale and scope of these events requires the availability of comprehensive analytical testing, which is currently lacking due to the speed with which new compounds appear. From a laboratory standpoint, the challenges of remaining current with synthetic cannabinoids stem from the diversity of compounds in the class, the large number of analogs and configurations, and delays in availability of analytical standards.

For the extraction of synthetic cannabinoids and their metabolites, blood samples (0.5mL) were prepared using liquid-liquid extraction with Tris hydrochloride buffer (pH 10.2) and MTBE. Urine samples (1mL) were prepared using Solid Phase Extraction (SPE) with ammonium carbonate buffer (pH=9.3) and elution with formic acid in methanol. All sample extracts were evaporated to dryness and reconstituted in 200µL of mobile phase.

Samples were acquired using a SCIEX® TripleTOF® 5600+ quadrupole TOF/MS coupled to a Shimadzu® Nexera® ultra high-performance liquid chromatograph. A reverse phase gradient using ammonium formate (10mM, pH 3) and methanol/acetonitrile (50:50) was employed for chromatographic separation on a Phenomenex® Kinetex® C18 analytical column (50mm x 3.0mm, 2.6µm) at a flow rate of 0.5mL/min. The total run time for analysis was seven minutes. Precursor ions were acquired by TOF/MS scan (100m/z–550m/z) via positive electrospray ionization. Precursor isolation was performed using SWATH® acquisition with overlapping windows from 10-25 Da in width, and fragmentation was achieved using a rolling collision energy of 35eV±15eV.

Data processing was performed using PeakView software with an Extracted Ion Chromatogram (XIC) list containing 262 synthetic cannabinoid parent compounds, metabolites, and internal standards. New standards are continually being added to the library as they become available. All previously analyzed casework data files are reprocessed using PeakView to investigate if other synthetic cannabinoids are present that were not known about at the time of initial testing.

This synthetic cannabinoid screening method was qualitatively validated for 19 parent compounds in blood and 19 metabolites in urine. These compounds were determined to be representative of the overall library database ( $n=262$ ), spanning a range of generations and diverse chemistries based on standard availability, and reflected the most currently prevalent drugs and their metabolites. Following three days of qualitative validation, all analytes met method validation performance criteria for precision/accuracy, limits of detection, interferences, processed sample stability, and carryover.

To date, 227 extracts from NMS Labs have been analyzed for synthetic cannabinoids. In total, 44 extracts were positive for at least one synthetic cannabinoid (parent or metabolite) for a positive rate of 19.4%. Table 1 shows all positive parent compounds identified and their rates of occurrence. This initial analysis has resulted in the identification of newly emergent synthetic cannabinoids, including 4-cyano-CUMYL-BUTINACA, 5F-EDMB-PINACA, and 5F-MDMB-PICA, which were not previously included in the scope at the time of testing.

**Table 1: Synthetic Cannabinoid Parent Positivity (June and July 2018)**

Parent Compound	Occurrence
5F-ADB (5F-MDMB-PINACA)	20
MMB-FUBINACA (FUB-AMB)	9
ADB-FUBINACA	8
5F-MDMB-PICA	6
AB-PINACA	2
4-cyano-CUMYL-BUTINACA	2
5F-EDMB-PINACA	2
5F-PB-22	1
AB-CHMINACA	1
5F-AMB	1

A rapid method for analysis of synthetic cannabinoids has been developed, validated, and successfully applied to forensic sample extracts. Additional populations, such as corrections and emergency room patients, are also being tested. As new synthetic cannabinoids appear on the market, they will be added to the library database and all previously acquired data will be re-interrogated for the new analyte. Data will be continually tabulated to create timelines and trend reports within these populations.

### Synthetic Cannabinoids, LC/qTOF, 5F-ADB



### **K48 Sample Mining: The Identification of Emerging Novel Psychoactive Substances (NPS) Through Reanalysis of Biological Extracts From Forensic Toxicology Casework**

Alex J. Krotulski, MS\*, Center for Forensic Science Research & Education, 2300 Stratford Avenue, Willow Grove, PA 19090; Susan Varnum, PhD, Temple University, 1801 N Broad Street, Philadelphia, PA; Barry K. Logan, PhD, NMS Labs/Center for Forensic Science Research & Education, 3701 Welsh Road, Willow Grove, PA 19090

**Learning Overview:** After attending this presentation, attendees will understand the value of real-time sample analysis and retrospective data analysis for the identification of emerging NPS present in toxicological extracts, but not within the initial scope of analytical testing.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing novel workflows and approaches for the identification of emerging NPS and providing information regarding NPS currently being detected, or undetected, in forensic casework.

NPS continue to pose health and safety threats to drug users and various clinical, forensic, public health, and public safety communities within the United States. Medical and forensic toxicologists are now tasked with the difficulty of interpretation of clinical and analytical findings, but this interpretation is dependent on the scope of testing, the availability of standard reference material, and the ability to characterize novel substances. This study sought to evaluate innovative methodologies for the identification of emerging NPS timely to first incident or detection using an NPS-rich dataset.

Discarded sample vial extracts from a large forensic toxicology laboratory (NMS Labs) were acquired for re-analysis against an extensive in-house library database. Sample extracts were collected from initial testing procedures for the directed analysis of select novel stimulants, novel opioids, and designer benzodiazepines, as well as other drug classes. Sample extracts were de-identified prior to inclusion in this study.

In-house re-analysis of sample extracts was performed via Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) using a SCIEX™ TripleTOF™ 5600+ coupled with a Shimadzu® Nexera® XR UHPLC. Chromatographic separation was achieved by a standard reverse phase gradient using ammonium formate (10mM, pH 3), methanol/acetonitrile (50:50), and a Phenomenex® Kinetex C18 analytical column (50mm x 3.0mm, 2.6µm). The total runtime was 15.5 minutes. Precursor ion mass acquisition was achieved by TOF/MS scan from 100-510 Da. Product ion mass acquisition was achieved following isolation using SWATH™ acquisition. Fragmentation occurred using a rolling collision energy of 35eV±15eV for the generation of mass-window specific fragment ion spectra. Resulting datafiles were compared against an extensive in-house library database containing precursor ion exact mass, retention time, fragment ion accurate masses ( $n=5$ ), and library mass spectra for more than 700 drugs, including a clear majority of NPS.

To date, 2,136 sample extracts have been re-analyzed using the described workflow. Comprehensive data processing has resulted in the identification of a wide-variety of NPS across several categories, some of which were incorporated into initial testing procedures. The most common novel opioids detected included cyclopropylfentanyl, methoxyacetylfentanyl, and fluoro-isobutyrylfentanyl; the most common novel stimulant detected was N-ethyl pentylone; and the most common designer benzodiazepines detected were flubromazepam and etizolam.

This novel workflow has resulted in the identification of NPS for the first time in toxicological specimens, including isopropyl-U-47700 and 3,4-methylenedioxy-U-47700. In addition, novel opioid precursors have been identified in the extracts, including N-methylnorfentanyl, benzylfentanyl, despropionyl-ortho-methylfentanyl, despropionyl-3-methylfentanyl, and benzylfurylfentanyl. While many of these fentanyl analogue precursors are believed to be inactive, their presence in toxicological extracts can provide useful information to pinpoint a likely route of manufacture or possibly novel opioid active agent.

NPS continue to emerge on the drug market. Based on the results of this study, biological extracts for sample mining and archived datafiles for data mining proved to be a rich dataset for the identification and discovery of emerging NPS. Forensic laboratories not currently utilizing updated broad-based screening methodologies or retrospective data analysis workflows should be aware that NPS in toxicological specimens could go undetected.

#### **NPS, Novel Opioids, Isopropyl-U-47700**



## K49 Fringe Analogs: The Emergence of New Synthetic Drugs in Postmortem Cases in Miami

Elisa N. Shoff, BS\*, Miami-Dade Medical Examiner Department, Miami, FL 33136; George W. Hime, MS, Miami-Dade Medical Examiner Department, Miami, FL 33136; Diane Boland, PhD, Miami-Dade Medical Examiner Department, Miami, FL 33136

**Learning Overview:** After attending this presentation, attendees will better understand new synthetic compounds that are becoming more prevalent in postmortem casework, specifically in Miami-Dade County, FL. Attendees will also gain an understanding of the difficulty in detecting these compounds, especially if they are not normally targeted in traditional drug screens.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing novel compounds that for the first time have been implicated in the cause of death in decedents in Miami-Dade County and Collier County, FL. Furthermore, this presentation will inform the forensic science community of the potential increase in dissociative hallucinogens and analogs of methylphenidate as drugs of abuse.

Since 2011, the Miami-Dade County Medical Examiner Department (MDME) has been detecting synthetic drugs in postmortem cases, specifically synthetic cathinones, cannabinoids, benzodiazepines, and most importantly, fentanyl/opioids. In recent years, analogs of compounds not frequently abused in Miami, such as arylcyclohexylamines (ketamine, phencyclidine) and methylphenidate, have emerged throughout the United States. To improve the detection of these various fringe analogs, the MDME has implemented a targeted drug screening/confirmation method into routine postmortem casework using an Ultra High-Performance Liquid Chromatograph (UHPLC), coupled to an Ion Trap-Mass Spectrometer (Ion Trap-MS). This data-dependent method utilizes a Scheduled Precursor List (SPL), retention time matching, and an in-house library built with certified reference materials for the purposes of identification. The SPL contains both a targeted molecular ion and daughter ion for each compound for the purposes of MS<sup>2</sup> and MS<sup>3</sup> spectral fragmentation. In addition, all library entries contain a full-scan MS, MS<sup>2</sup>, and MS<sup>3</sup> spectral profile for each compound.

Currently, three analytes that can be classified as fringe analogs—3-Methoxy-PCP, 4-Methoxy-PCP, and 4-Fluoromethylphenidate—were added to a previously validated method that targets designer analogs. Limits of detection in extracted whole blood were established for these three analogs, at 0.5ng/mL, 1ng/mL, and 1ng/mL, respectively. All case samples analyzed using this method were postmortem whole blood specimens, extracted using mixed-mode solid phase extraction columns. All case samples were initially screened on a Gas Chromatograph coupled to a Mass Selective Detector (GC-MSD). Since the beginning of 2018, three cases screened positive for at least one of the three fringe analogs added into the targeted method. Further detail of the analog detected, and other case information, is shown in the table below.

Case #	History	Analog(s) detected via LC-Ion Trap-MS	Analog(s) detected via GC-MSD	Cause of Death	Manner of Death
1	Found unresponsive in bed by family members. Had history of illicit drug use.	3-Methoxy-PCP	3-Methoxy-PCP	Multiple Drug Toxicity (3-Methoxy-PCP included)	Accident
2	Unrestrained driver that collided with a bus. Had used illicit drugs in the past.	3-Methoxy-PCP	None	Blunt Injuries of Torso	Accident
3	Found unresponsive. History of illicit drug use.	3-Methoxy-PCP, 4-Fluoro methylphenidate	4-Fluoro methylphenidate	Pending Toxicology	Pending

Because potency and potential toxic concentrations are unknown for these compounds, sensitive and targeted methods are ideal. The increased presence of these new synthetic drugs has led to the development of additional methods at the MDME, both for the purposes of quantifying these compounds, and adding other related analogs to the current UHPLC-Ion Trap-MS targeted screening method. With further studies and testing, more definitive conclusions can be drawn from this handful of unique compounds emerging in the Miami synthetic drug scene.

### 3-Methoxy-PCP, 4-Fluoromethylphenidate, Postmortem Toxicology



## K50 A Carfentanil Outbreak in Florida in 2016

*Chris Delcher, PhD, University of Florida College of Medicine, Gainesville, FL 32608; Yanning Wang, MS, University of Florida, Gainesville, FL 32610; Russell S. Vega, MD, District 12 Medical Examiner's Office, Sarasota, FL 34239; Jessica Hvozdoch, BS\*, University of Florida, Gainesville, FL 32608; Chris W. Chronister, PhD, University of Florida, Gainesville, FL 32608; Bruce A. Goldberger, PhD, University of Florida College of Medicine, Gainesville, FL 32608*

**Learning Overview:** After attending this presentation, attendees will understand the sociodemographic information, geographic and temporal patterns, and prescription histories associated with a fatal overdose outbreak caused by the introduction of carfentanil to a local illicit drug supply. Deaths concentrated in the Sarasota, FL, area were compared to other carfentanil-involved fatal overdoses in Florida from June to December 2016 as this synthetic opioid spread throughout the state.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by sharing outbreak details and warning signs for more targeted, rapid responses to emerging novel psychoactive substances. Illicitly manufactured fentanyl analogs pose a serious public health threat and have changed the way the opioid overdose epidemic must be approached in the United States. Accelerated reporting, reliable data sharing systems, and proactive pharmacovigilance are needed when combating this dynamic crisis.

**Methods:** Data sources for this study included the Florida drug-Related Outcomes Surveillance and Tracking (FROST) System and the Electronic-Florida Online Reporting of Controlled Substance Evaluation Program (E-FORCSE), which acts as the Florida Prescription Drug Monitoring Program (PDMP). Date-of-death information was provided by the District 12 Medical Examiner's Office. Descriptive statistics, chi-square tests, and epidemic curves compared characteristics of the Sarasota area with the remainder of the state.

**Results:** Of the 548 statewide carfentanil deaths in 2016, 114 (20.8%) occurred in the Sarasota area, a region that only accounts for 4.0% of Florida's population. The Sarasota overdose epidemic began with four deaths in June 2016, peaked with 37 deaths in July, and declined substantially by November. In July, more carfentanil deaths occurred in this area than the rest of the state combined ( $n=17$ ). The height of the outbreak did not occur for the rest of Florida until October (137 deaths, 25.0% of the state total), demonstrating an approximate two-month lag. Most decedents in Sarasota were White males (73.7%) and over one-third of all decedents were between 25 and 34 years old. Concomitant drugs in both Sarasota and the rest of Florida included cocaine (50.9%), oxycodone (9.6%), and alprazolam (23.7%). However, carfentanil-positive decedents in Sarasota were less likely to test positive for heroin (7.9% v. 23.5%), fentanyl (7.0% v. 22.6%), and other fentanyl analogs (1.8% v. 25.8%). According to statewide PDMP data, at least one controlled substance was dispensed within two years prior to death in 94 decedents (82.5%), and half ( $n=47$ ) filled the prescription within one year of death. Approximately 21.1% of decedents had no or insufficient evidence of a prescription.

**Discussion/Conclusion:** Timing and concomitant substance patterns suggest Sarasota was a point of entry for carfentanil in the state of Florida. This study demonstrates that rapid, geographically targeted responses are necessary to mitigate similar incidents during their early stages and additional steps are necessary to prevent similar outbreaks in the future. For example, Florida medical examiners now have access to the state PDMP, strengthening the medicolegal death investigation process with clinical data, for improved surveillance and response to fatal overdoses. However, this incident demonstrated that additional efforts are needed to determine how pharmacovigilance signals, such as unusual prescribing patterns, can initiate early intervention. Further, new "listening platforms" (e.g., drug-focused social media) and data systems (e.g., emergency medical services transports) may assist authorities with the rapid identification of emerging drugs in communities. In this incident, law enforcement and the media were the first entities to caution the public of the carfentanil outbreak in Florida, emphasizing the need for local, interagency data-sharing and more effective, standardized communication among stakeholders. The forensic toxicology backlog and the resulting delay in cause-of-death reporting by medical examiners are often the rate-limiting steps for such communication. Establishing routine methods for fentanyl analog analysis and identifying novel substances remains a significant challenge for laboratories across the country. The Centers for Disease Control and Prevention's (CDC's) Enhanced State Opioid Overdose Surveillance (ESOOS) Program provides funding to support more timely and comprehensive data acquisition, including toxicology for both non-fatal and fatal opioid overdoses, as well as the assessment of risk factors associated with fatal overdoses. In the future, these data will alert public health and safety organizations to changes in the illicit drug market that have the potential to develop into fatal outbreaks, facilitating efforts for more effective responses.

### Carfentanil, Epidemiology, Fatal Drug Overdoses





**K51 Examining Demographics and Emerging Drug Trends on Accidental Deaths Due to Intoxication in Washington, DC**

*Samantha Tolliver, PhD\*, Office of the Chief Medical Examiner, Washington, DC 20024; Lucas W. Zarwell, MFS, Washington, DC 20011*

**Learning Overview:** After attending this presentation, attendees will understand how the proliferation of laced heroin has impacted intoxication deaths from 2013 to 2017 within the District of Columbia.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by examining drug trends and the demographic information of the decedent population within the District of Columbia.

At the District of Columbia Office of the Chief Medical Examiner (DC OCME) between the years of 2003 and 2012, the combination of natural (55%) and accidental (25%) deaths accounted for 80% of the case load. On average, accidental deaths were largely comprised of blunt injury, followed by intoxication. In the order of prevalence, cocaine, ethanol, and heroin were routinely detected among those cases. However, fentanyl was not commonly detected at that time, as it was only listed in 2010, as being identified in 3% of the cases. Fentanyl reappeared in 2013, when it was identified in 1% of the accidental death cases. This presentation will focus on accidental deaths that were specifically due to drug intoxications between the years of 2013 and 2017.

Table 1 lists the most commonly encountered analytes and/or analyte classes identified within the accidental drug intoxication population in the District of Columbia from 2013 to 2017. A review of the data indicated that deaths attributed to heroin usage were the most prevalent. Within that time, heroin deaths peaked in 2015, which is the same year that fentanyl analogs (4%) began to emerge within the decedent population. An examination of the race and gender demographics (Table 2) revealed that, on average, those most impacted by accidental drug intoxications were Black males (57%); followed by Black females (23%), White males (13%) and White females (3%).

Year	Cocaine & metabolites	Heroin & metabolites	Ethanol	Opioids	Fentanyl	Fentanyl Analogs	PCP
2013	25.34%	26.03%	15.75%	9.59%	1.37%	0.00%	8.22%
2014	22.86%	23.33%	15.71%	13.81%	3.81%	0.00%	4.76%
2015	14.39%	32.20%	15.15%	9.09%	7.58%	4.55%	2.65%
2016	15.37%	22.09%	14.78%	7.31%	13.88%	8.51%	6.27%
2017	16.39%	17.45%	12.03%	4.48%	20.05%	9.43%	4.13%

**Table 1:** Commonly Encountered Drugs in Accidental Intoxication Deaths between 2013–2017

Year	Black Male	Black Female	White Male	White Female
2013	54.64%	24.74%	17.53%	1.03%
2014	50.43%	24.35%	18.26%	4.35%
2015	53.33%	21.33%	20.00%	5.33%
2016	64.38%	18.30%	10.78%	2.61%
2017	56.59%	27.47%	9.07%	3.57%
Average	57.34%	23.17%	12.93%	3.38%

**Table 2\*:** Race and Gender Demographics from 2013–2017

\* All other race/ gender combinations that averaged less than 2% of the cases were excluded.

When compared to 2015, 2016 saw a decrease in the detection of heroin. However, this was met with an increase in fentanyl and its analogs. As a result, Black males, who accounted for 64% of the accidental intoxications, reached a record high within this cause of death. Conversely, Black females, White males, and White females all saw decreases within their populations. This seems to be in stark contrast to the demographic information generated throughout the United States, as reportedly White adults (aged 25–54 years old) are those most affected by the opioid crisis.<sup>1,2</sup>

As compared to 2016, 2017 continued the pattern of decreased identification of heroin with an increase in fentanyl and its analogs. Cases containing any combination of heroin, fentanyl, or fentanyl analogs accounted for nearly half (46%) of the intoxication deaths in the District of Columbia. Although Black males saw a 12% decrease in intoxication deaths during this time, they remained the most affected demographic. At 27%, Black females had their largest amount of intoxication deaths (range: 18%–27%) for the years examined. Conversely, accounting for 9% of these cases, White males had their lowest (range: 9%–20%) amount of intoxication deaths. White females, at 3% (range: 1%–5%), had the least amount of intoxication deaths among the race/gender demographics with 2% or more of the cases.



The impact that fentanyl and its analogs have had on drug deaths cannot be denied. The District of Columbia has experienced record highs among its intoxication deaths, as well as within the Black male population. These increases moved in parallel with the proliferation of laced heroin, thus further emphasizing how critical it is for a forensic toxicology laboratory to be able to provide analytical testing that is in line with the current drug market.

**Reference(s):**

1. <https://www.cdc.gov/drugoverdose/pdf/pubs/2017-cdc-drug-surveillance-report.pdf>.
  2. <https://www.whitehouse.gov/opioids/>.
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**Demographics, Fentanyl, Drug Trends**



## K52 Long-Term Phencyclidine (PCP) Usage Trends in the District of Columbia

Kristin Jones, BS, Egg Harbor Township, NJ 08234; Charis Wynn, MSc\*, Office of the Chief Medical Examiner, Washington, DC 20024; Lucas W. Zarwell, MFS, Washington, DC 20011

**Learning Overview:** After attending this presentation, attendees will understand: (1) PCP use specific to the District of Columbia population based on a retroactive study observing poly-drug use and sample concentrations, and (2) the effects of PCP based on a review of published literature.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by serving as a statistical resource on how the longstanding use of PCP has historically affected the community. Nationally, PCP is not a common drug of abuse and is often overlooked in trend reports. However, it is among one of the most prevalent illicit drugs abused specific to the District of Columbia.

PCP was developed in the 1950s as an intravenous anesthetic for both human and veterinarian use. It is known illicitly under a variety of names such as Angel Dust; is found in many forms, such as powder, crystal, tablet, and liquid; and can be injected, snorted, ingested, or smoked. PCP is a non-competitive antagonist for N-Methyl-D-aspartic (NMDA) receptor resulting in the high variance in effects observed in users. PCP's continuous resurgence in the community is possibly a result of a phenomenon called "generational forgetting" in which there was a continuous flow of new drugs onto the scene and older ones being rediscovered and reinvented by younger generations, such as combining phencyclidine with marijuana.

A retrospective study on Driving Under the Influence (DUI) and postmortem cases at the District of Columbia Office of the Chief Medical Examiner (OCME) were conducted to demonstrate and draw conclusions regarding trends. This study reviewed 145 postmortem cases from 2003 to 2017 in which PCP was listed in the cause of death, and 868 DUI cases from 2010 to 2017 in which PCP was present. Data was generated based on key components of the cases, such as poly-drug use and sample concentrations. Statistics were analyzed to determine correlation in specimen source, antemortem and postmortem concentrations, and any increasing or decreasing trends over the years.

The number of postmortem cases in which PCP was listed increased from 2003 to 2017 by a factor of ten. Manner of death in the 145 PCP postmortem cases were mostly accidental with only five homicides, three of which were while in police custody. The most common drugs, in order of prevalence, used with PCP in postmortem poly-drug cases were cocaine, ethanol, and heroin. The number of DUI cases in which PCP was present increased from 2010 to 2017 by 14%. The most common drugs, in order of prevalence, used with PCP in DUI poly-drug cases were marijuana, benzoylcegonine, and codeine. There was no correlation between femoral, heart, or hospital blood PCP concentrations for postmortem or antemortem cases. Table 1 compares the findings within the District of Columbia population to that of established literature:

	Postmortem Blood	Antemortem Blood (DUI)
Reference <sup>1</sup> Average	1.1mg/L	0.075mg/L
Reference <sup>1</sup> Range	0.5-3.3mg/L	0.007-0.240mg/L
In-house Average	0.072mg/L	0.023mg/L
In-house Range	0.01-0.37mg/L	0.01-0.15mg/L

**Table 1:** Phencyclidine Concentrations in Postmortem and Antemortem Blood

Over the past 15 years, the District of Columbia has seen an increase in PCP postmortem cases, possibly due to a policy change that increased reporting and decreased inconsistencies with listing specific drugs in case reports. Generally, over the past seven years, PCP prevalence in DUI casework has increased. Regionally, our concentrations across postmortem and antemortem populations tend to be lower than what is suggested in literature sources surveying other areas. When analyzing OCME casework from 2003 to 2017, data demonstrates that accident cases are most likely the cause of death associated with most PCP-related postmortem cases. However, there is no correlation in blood concentration of PCP use and the manner of death. At higher doses of PCP, users tend to be more aggressive and display increased strength, which possibly explains homicide cases accounting for the second most common cause of death associated with PCP intoxication due to restraints in police custody.

### Reference(s):

<sup>1</sup>. Baselt, Randall C. *Disposition of Toxic Drugs and Chemicals in Man*. 11<sup>th</sup> edition. 2017.

### Phencyclidine, Retroactive Study, District of Columbia



### K53 Determining the Prevalence of Gabapentin Abuse Among Opioid Users in Northern Virginia

Alexa Genalo, MSc\*, Fairfax, VA 22030

**Learning Overview:** The goal of this presentation is to provide insight on the specific trend of gabapentin abuse among opioid users in the Northern Virginia region.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing new information on a specific drug trend in Northern Virginia directly related to the ongoing opioid crisis in the United States. The research covered in this presentation complements similar studies that have been previously conducted and proves gabapentin is an abused substance that should be federally controlled.

Gabapentin is an Anti-Epileptic Drug (AED) used in the treatment of epilepsy and postherpetic neuralgia. It was first approved for use in 1993 and is not a scheduled drug under the Controlled Substances Act due to its perceived low abuse potential and little to no drug-drug interactions. Today, gabapentin is also used off-label to treat a variety of conditions, including chronic pain and substance-dependence disorders. More often than not, gabapentin is prescribed to patients in conjunction with opioid compounds to treat their chronic pain. However, recent studies have shown that when this combination of drugs are used together, gabapentin enhances the effects of the opioid compounds, leading to increased levels of sedation and respiratory depression. These heightened effects have led to a trend of abuse of gabapentin with both licit and illicit opioid compounds across the United States. This study serves to explore the presence of this gabapentin abuse among opioid users in the counties of Northern Virginia serviced by the Virginia Department of Forensic Science (VA DFS) Northern Laboratory.

The goals of this research were to establish the presence of gabapentin in opioid-positive samples, to determine the percentage of gabapentin-positive cases, and to examine the gabapentin-positive cases for the type of opioid compounds present to determine whether illicit or prescription opioids were used more. For the purposes of this research, illicit opioid compounds were defined as heroin, fentanyl, and fentanyl derivatives. It was hypothesized that gabapentin would be present in a high percentage of opioid-positive samples and that gabapentin would be abused more commonly with illicit opioid compounds.

Blood samples received by the Toxicology Section of the VA DFS Northern Lab that were confirmed to contain opioid compounds were tested for the presence of gabapentin using a liquid-liquid extraction method and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) instrumentation. Chromatograms collected from the instrument were analyzed to determine the presence or absence of gabapentin in the selected samples. Gabapentin was present in 9% of the total number of samples tested. There was insufficient data to determine whether prescription or illicit opioids were used with gabapentin more, as the gabapentin-positive samples contained both prescription opioid compounds and illicit opioid compounds. The results of this study demonstrate the presence of this drug trend within our community and complement similar studies conducted on a broader scale in proving that gabapentin has an abuse potential and should be a federally controlled substance.

#### Gabapentin Abuse, Opioids, Anti-Epileptic Drugs



## K54 The Use of Molecular Modeling to Investigate the Influence of Structural Characteristics on the Binding of Synthetic Cannabinoids to the CB1 Receptor

Caroline Spencer, BS\*, Oxford, MS 38655; Pankaj Pandey, PhD, University of Mississippi, University, MS 38677; Robert J. Doerksen, PhD, University of Mississippi, University, MS 38677; Murrell Godfrey, PhD, University of Mississippi, University, MS 38677

**Learning Overview:** After attending this presentation, attendees will better understand the interactions between synthetic cannabinoids and the CB1 receptor and how structural changes of these compounds influence the interactions taking place within the binding pocket.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by contributing to the understanding of essential interactions between specific substituents of synthetic cannabinoids with CB1 receptor residues through molecular modeling. The knowledge of these key interactions can help lead to a better understanding of the effects of these compounds and aid in the discovery of possible new synthetic cannabinoids.

Synthetic cannabinoids have emerged onto the drug scene as an alternative to illegal marijuana.<sup>1</sup> Like  $\Delta$ -9 Tetrahydrocannabinol ( $\Delta$ -9 THC), the main psychoactive ingredient in marijuana, synthetic cannabinoids interact with G-coupled protein receptors found in the brain, immune system, and peripheral organs.<sup>2</sup> There have been two cannabinoid receptors identified—CB1 and CB2. The binding of THC and synthetic cannabinoids to the CB1 receptors located in the brain is believed to induce the psychoactive effects. Even though there are similarities between  $\Delta$ -9 THC and synthetic cannabinoids, many synthetic cannabinoids have been determined to be full agonists for the CB1 receptor whereas  $\Delta$ -9 THC is only a partial agonist.

In this study, an active-state CB1 receptor model, proposed by the Doerksen lab, was used to compare the ligand-receptor interactions between the JWH synthetic cannabinoids, developed by John W. Huffman, and the CB1 receptor.<sup>3</sup> This study focused on the naphthylindole group of JWH synthetic cannabinoids. This group includes JWH-007, 018, 073, 116, 122, 182, and 210. This study was accomplished using Schrödinger's Maestro molecular modeling software. JWH ligands were selected based on their affinity for binding to the CB1 receptor. The docking of the ligands to the receptor took place after both the ligands and CB1 receptor model were prepped for docking and a grid of the active site was generated. Parameters were set to give the five best possible poses, or positions, for the ligands. Once the ligands were docked to the CB1 receptor model, the interactions were thoroughly analyzed. The information collected from this study includes the amino acid residues interaction with the ligands and the bond distances of these interactions, the docking score of each ligand and each pose, estimated binding affinities, and ligand  $K_i$  values. Once the known synthetic cannabinoids were analyzed, new structures were designed based on specific structural characteristics. The new structures were studied in the same way as the known synthetic cannabinoids to determine how these structural changes influence the binding and interactions of these compounds to the CB1 receptor.

The results of this study show the influence structural characteristics have on the docking score and relative binding affinity of the synthetic cannabinoids to the CB1 receptor. One structural characteristic examined in this study was the increasing alkyl chain length on the naphthalene group present in these ligands. The results were able to show a trend in docking score when increasing the alkyl chain length by one carbon on the naphthalene from no alkyl chain to a pentyl chain. The trend in docking scores was also shown to be dependent on the length of the alkyl chain (butyl or pentyl) that was on the nitrogen of the indole portion of the compound. However, when the structural characteristic of the carbonyl group was removed from the structures, the trend in docking scores was found to be reversed. A correlation was also seen with the docking score trend and the number of aromatic stacking interactions taking place in the binding pocket. Examining the JWH ligands docking revealed key interactions that were seen consistently in the active site. These interactions included Trp 279, Trp 356, Phe 177, and Phe 200. Identifying the key interactions between the synthetic cannabinoids and the CB1 receptor, and how their structural components influence the interactions, can lead to a better understanding of the effects of these drugs, including toxicity and potential for abuse. Future research will include the development of a database consisting of known synthetic cannabinoids, as well as newly designed synthetic cannabinoids, and pharmacological information of these compounds to aid in the rapid identification and understanding of synthetic cannabinoids as they appear in the drug market.

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3. Vardakou, I. et al. Spice Drugs as a New Trend: Mode of Action, Identification and Legislation. *Toxicology Letters*, vol. 197, no. 3, Jan. 2010, pp. 157-162., doi:10.1016/j.toxlet.2010.06.002.

### Synthetic Cannabinoid, CB1 Receptor, Molecular Modeling



### K55 Can Synthetic Urine Replace Authentic Urine to Cheat Drug Testing?

*Victor J. Kim, BBA\*, Tripler Army Medical Center, Honolulu, HI 96859; Catherine K. Okano, PhD, Tripler Army Medical Center, Tripler Army Medical Center, HI 96859; Caroline Osborne, BS, Tripler Army Medical Center, Honolulu, HI 96859; Deanna Frank, BS, Tripler Army Medical Center, Honolulu, HI 96859; Christopher Meana, BS, NCIS Hawaii Field Office, Honolulu, HI ; Marisol Castaneto, MS, Ewa Beach, HI 96706-6430*

**Learning Overview:** After attending this presentation, attendees will understand what Synthetic Urine (SU) is, if it can really beat workplace drug testing, and its negative effect to our community and society.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that drug testing can be fooled with the substitution of SU products, which could bring about meaningful changes to make workplace drug testing as effective as possible.

**Background:** Urine is still the most common matrix utilized in workplace drug testing. Workplace drug testing in the United States Department of Defense was established to deter service members from illicit drug use and ensure personnel readiness. Federal civilian employees in drug testing positions also undergo Urine Drug Testing (UDT) before and during employment. Attempts to obscure illicit drug use have been observed in UDT programs with excessive hydration, urine adulterants, and urine substitutions with or without the use of prosthetic sex part (e.g., “Whizzinator”). SU is utilized by drug testing laboratories as a matrix for quality control preparations. However, SU products are now commercially sold as “novelty” and/or “fetish urine.”

**Objective:** To determine if SU can pass as authentic urine, this study challenged Army UDT collection and testing procedures using eight different commercial SUs.

**Material and Methods:** Eight SU products purchased from local smoke shops or online vendors were poured in plastic collection urine bottles. Adulterated/substituted samples such as 10% bleach in water (1:5 dilution), 10% bleach in urine (1:5 dilution), 10% hydrogen peroxide in urine (1:5 dilution), apple cider vinegar, and apple juice were also individually poured in collection bottles. These samples were placed randomly in boxes with other negative urine specimens. Three laboratory technicians were instructed to process the samples and pour sample aliquot to undergo immunoassay screening per standard operating procedures. Aliquots were screened in the Beckman AU5800 analyzers for cannabinoids, cocaine, amphetamines, codeine/morphine, hydrocodone/hydromorphone, oxycodone/oxymorphone, benzodiazepines, and synthetic cannabinoids. All SU, five adulterated urine samples, and two donor urine specimens were sent to a separate drug testing laboratory for specimen validity testing. None of the technicians in either laboratory had prior knowledge of which were adulterated or SU samples. Additionally, on-site SU (Synthetic UrineCheck™) and adulteration strips (Sciteck® AdultaCheck® 6) provided by Sciteck® were challenged with SU, adulterated urine, and negative authentic urine. Water and donor urine samples were used as controls for the Synthetic UrineCheck™. The Sciteck® AdultaCheck® 6 strips tested for oxidant, creatinine, nitrite, glutaraldehyde, pH, and chromate.

**Results:** Drug laboratory personnel identified five of eight SU products during accessioning. All SU products screened negative in the drug immunoassay and additionally passed the specimen validity testing as valid urine. The SU detection test strips were able to discriminate between authentic urine and SU products, although the test strip was not able to distinguish between diluted urine and SU. When the diluted urine and SU samples were tested with the Sciteck AdultaCheck® 6, all passed the adulteration test strips and were identified as authentic urine.

**Conclusion:** Direct observation during the collection process is critical to deter donors from adding or substituting their own urine specimens. In the civilian UDT, temperature strips may be affixed to the collection bottles or placed on the bottles immediately after collection to measure urine temperature. The specimen’s temperature must read between 90°F–100°F (32°C–38°C). In contrast, military UDT do not use temperature strips and rely on randomly selected observers to monitor any foul play during the collection process. In either circumstance, the use of “Whizzinator,” a prosthetic penis, has become an option for drug users. The prosthetic penis is attached to a bladder with a temperature strip. To deter SU use, UDT programs need to enforce optimal environment and direct observation during the collection process.

#### Synthetic Urine, Workplace Drug Testing, Urine



### K56 Detection of *In Utero* Ethanol Exposure Via Ethyl Glucuronide and Ethyl Sulfate Analysis in Placenta

Jennifer Hanna, New York, NY 10019; Ana De Castro, PhD, Universidade de Santiago de Compostela, Santiago de Compostela 15782, SPAIN; Elena Lendoiro, MD, University of Santiago de Compostela, Santiago de Compostela 15782, SPAIN; Angelines Cruz-Landeira, PhD, MD, Universidade de Santiago de Compostela, Santiago de Compostela 15782, SPAIN; Manuel Lopez-Rivadulla, PhD, University of Santiago de Compostela, Santiago de Compostela 15782, SPAIN; Marta Concheiro-Guisan, PhD\*, John Jay College of Criminal Justice, New York, NY 10019

**Learning Overview:** This presentation will demonstrate to attendees the value of utilizing placenta as an alternative matrix to meconium for the determination of *in utero* ethanol exposure, and the extent to which the two matrices can be compared.

**Impact on the Forensic Science Community:** This research will impact the forensic science community by providing a sensitive and specific method employing an alternative matrix to improve the interpretation of *in utero* ethanol exposure that will help toxicologists, legal personnel, and clinicians understand the newborns' outcomes and the medical and legal consequences of this exposure.

According to the 2016 National Survey on Drug Use and Health, ethanol was the second most prevalent substance (8.3%) after tobacco (10%) among pregnant women in the United States, with the ethanol prevalence being higher among 26- to 44-year-old pregnant women than in the 18- to 25-year-old group (9.1% vs. 6.5%). Alcohol consumption during pregnancy may happen due to preconceived notions among the population that a minor amount of alcohol during pregnancy can be harmless. However, there is no amount of ethanol deemed safe for consumption during pregnancy. Alcohol exposure during pregnancy constitutes one of the leading preventable causes of birth defects, mental retardation, and neurodevelopmental disorders in exposed children. Objective analytical methods are necessary to monitor this exposure.

A method for the determination of *in utero* ethanol exposure utilizing the direct minor metabolites of ethanol, Ethyl Glucuronide (EtG), and Ethyl Sulfate (EtS), has been developed, validated, and applied to authentic placenta samples from newborns whose meconium tested positive for EtG (>5ng/g). A 0.1g of placenta was mechanically homogenized in methanol and extracted using weak anion exchange solid phase cartridges and analyzed via Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Chromatographic separation was performed by Kinetex® Polar C18 column using a gradient elution of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Each compound was quantified and confirmed using its respective internal standard and two transitions in Multiple Reaction Monitoring (MRM) mode. Validation results demonstrated a linear range of 10ng/g–500ng/g with a limit of quantification of 10ng/g for both EtG and EtS, with residuals within  $\pm 20\%$  and a coefficient of determination greater than 0.99. Imprecision was maintained below 12%, and accuracy was within 83.1%–112.4%. Matrix effects (-69.9%– -15.2%), extraction efficiency (75%–92.7%), process efficiency (22.5%–76.3%), and interferences and autosampler stability at 10°C were also assessed during validation.

The validated method was applied to 59 authentic placenta samples. EtG and/or EtS was detected in 8 out of 59 samples with ranges of 26.5ng/g–266.5ng/g and 11ng/g–24.3ng/g, respectively. Results from placenta were compared to different EtG meconium cutoffs from the literature in paired samples to establish relative rates of agreement between the two matrices. The highest agreement of 50% was achieved at EtG cutoff of 444ng/g in meconium, and the lowest agreement of 13.6% at EtG analytical cutoff of 5ng/g in meconium.

A sensitive and specific method for the determination of EtG and EtS in placenta was developed. Comparing meconium and placenta, meconium showed a higher sensitivity, but placenta could be employed as alternative sample if meconium is not available. To date, this is the first study to compare placenta and meconium to detect ethanol exposure during pregnancy.

#### Ethanol, Ethyl Glucuronide, Ethyl Sulfate

## K57 Co-Reaction of Lactate/Lactate Dehydrogenase in an Enzymatic Ethanol Assay

Julia C. Liebl, BA\*, University of New Haven, West Haven, CT 06516; Robert H. Powers, PhD, University of New Haven, West Haven, CT 06516

**Learning Overview:** After attending this presentation, attendees will understand how an enzymatic ethanol assay can produce false positives or falsely elevated results for samples containing elevated levels of lactate and Lactate Dehydrogenase (LDH) due to trauma or illness. Attendees will also understand threshold levels of lactate and LDH necessary for interference to occur and the magnitude of potential effects on ethanol results.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by clarifying the potential for interference by lactate/LDH in an Alcohol Dehydrogenase (ADH)-based ethanol assay and providing an understanding of the physiological parameters that could reasonably cause such an interference. The results of this project can be used to facilitate the interpretation of enzymatic ethanol results by pathologists, medical personnel, and toxicologists.

Enzymatic assays are commonly used to determine blood alcohol content in clinical settings. In some instances, this data may be used as evidence in legal settings (e.g., Driving Under the Influence (DUI) cases). The enzymatic assay measures the amount of ethanol indirectly as  $\Delta$  NADH. It is theoretically possible that interference from the oxidation of lactate to pyruvate could occur and produce a falsely positive or elevated result.<sup>1</sup> While addressed sporadically over the past 30 years, questions of the conditions under which such interference may occur remain unresolved, particularly in the LDH range of 4,000U/L–10,000U/L. While abnormally elevated levels of lactate and LDH are not routinely present in clinical samples, nor are erroneous “false positive” ethanol results considered to be a common problem, there has been persistent concern regarding the potential for such interference affecting some samples of legal significance.<sup>2-9</sup> Further, no information regarding the combination of ethanol, lactate, and LDH in an ADH assay has been provided.

To determine if the presence of lactate and LDH in a serum sample could impact the ethanol level observed in the assay, varying amounts of lactate and/or LDH were added to an enzymatic ethanol assay. The concentrations of lactate and LDH utilized were chosen to reflect elevated levels reasonably observable in living individuals (normal lactate: 0.5mM–2.2mM, elevated lactate: 5mM–50mM; normal LDH: 122U/L–225U/L, elevated LDH: 200U/L–10,000U/L) suffering from a combination of trauma, shock, hypoxia, hepatic disease, or other conditions producing leakage of hepatic enzymes and lactic acidosis.<sup>5, 10,11</sup>

The results indicate that elevated levels of lactate and LDH can result in false positive and falsely inflated results from the ADH assay, most notably at higher concentrations. In a system with no ethanol, the lower threshold of response was detected with a combination of 4,000U/L LDH and 50mM lactate. This indicates that interference occurs far above physiologically normal levels of LDH and explains the rarity of this interference. On the upper end, 10,000U/L LDH and 50mM lactate produced a signal of 92mg/dL, which may have significant legal implications.

When lactate and LDH were added to a system with ethanol present, a signal increase of up to 350% was observed, with greater effects noted with low (30mg/dL) and medium (60mg/dL) levels of ethanol in the system. These results indicate that lactate/LDH interference, while rare, can conceivably cause a significant false positive or falsely inflated “ethanol” result.

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### **Enzymatic Ethanol Assay, Lactate Interference, False Positive**



## K58 The Application of Statistical Design of Experiments (DoE) to Assess External Decontamination Methods in Forensic Hair Analysis

Jennett M. Chenevert, MS\*, Florida International University, Miami, FL 33199; Anthony P. DeCaprio, PhD, Florida International University, International Forensic Research Institute, Miami, FL 33199

**Learning Overview:** After attending this presentation, attendees will understand how statistical DoE can help optimize highly variable forensic methodologies, particularly in forensic hair analysis, with the ultimate goal of working toward standardization of these techniques.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing that this work will contribute to a body of research aimed at determining the most effective method(s) for decontaminating hair samples for forensic analysis and subsequently isolating drugs of abuse from these samples.

It was hypothesized that statistical DoE could be used to determine which conditions of a discrete set of levels of defined parameters would result in the most effective removal of amphetamine from the surface of human head hair, deposited by means of external environmental exposure. As a complex solid sample matrix, hair requires pretreatment measures, including decontamination, homogenization, and extraction, to remove the drug from the hair matrix components to allow for analysis. Optimizing pretreatment parameters is essential to accurately identify and quantify drugs present in a hair sample. One of the most challenging issues faced in hair testing analysis and interpretation is the differentiation between a drug that is detected due to superficial deposition onto the surface of the hair and a drug incorporated into hair from intake and systemic distribution. This interpretive challenge necessitates effective removal of the drug on the surface of hair deposited by means of contamination from an individual's environment prior to analysis. The Society of Hair Testing (SoHT) recommends at least one wash step with an aqueous solvent and one with an organic solvent. These guidelines are general, and thus a variety of decontamination methods have been published to date without a systematic comparison of these different techniques.

The major goal of this work was to conduct a comprehensive DoE analysis of decontamination parameters and their effect on the removal of drug from the surface of hair. A 2<sup>4</sup>-fractional factorial block design followed by Analysis Of Variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) analysis were used to compare the main factors: wash time, aqueous and organic decontamination solvent identities, and order of wash (i.e., organic wash first vs. aqueous wash first). The blocking factors were the number of sequential washes with both the aqueous and organic solvents. Neat amphetamine powder was superficially applied to blank human head hair. The hair was decontaminated following the 2<sup>4</sup>-fractional factorial block design matrix.

A 30mg aliquot of hair was weighed into a stainless steel milling jar with 200µg of amphetamine sulfate. The samples were agitated using a BioSpec® Mini-Beadbeater™ 24 ball mill, without milling beads present. The samples were then washed with the aqueous or the organic solvent first for either 30s or 30min. The aqueous wash was performed by adding 1mL of chromatography-grade water or 1% Sodium Dodecyl Sulfate (SDS) and agitating on an orbital shaker. The organic wash was performed by adding 1mL of 2-propanol or dichloromethane and agitating on an orbital shaker. There were 1–3 washes of the aqueous and organic washes performed, depending on the two blocks of the experimental unit. The hair was then dried for 24h at 40°C in an oven. Once dried, the samples were homogenized using the ball mill with chrome-steel milling beads at 3,800rpm for 30s. The drug was extracted by means of enzymatic hydrolysis of the hair proteins, with a pre-incubation in 12mg/mL aqueous Dithiothreitol (DTT) for 2h at 37°C, followed by incubation with 2mg/mL proteinase K. Extracted samples and wash solutions were purified by spin filter centrifugation (3 KDa MW cut-off) and Solid Phase Extraction (SPE) before Liquid Chromatography/Triple quadrupole/Mass Spectrometry (LC/QqQ/MS) analysis. Three replicate samples for each design point were analyzed.

Analysis of Variance (ANOVA) results indicated that 3- and 4-factor interactions (i.e., the parameters associated with combinations of 3 and 4 parameters under study) were significant. The percent of drug recovered from the hair to which amphetamine was superficially applied was highest, at 46%, for samples that were washed for 30s during each wash step, employed dichloromethane as the organic wash solvent, and were washed with the organic solvent before the aqueous solvent. When expanded to additional drugs, it is expected that this work will contribute to a body of research aimed at determining the most effective method(s) for decontaminating hair samples for forensic analysis and subsequently isolating drugs of abuse from these samples.

### Forensic Hair Analysis, External Contamination, Statistical Design of Experiments



### K59 An Evaluation of a Presumptive Screen in Urine as a Rapid Method of Determining Fentanyl-Related Deaths

Aaron M. Shapiro, PhD\*, British Columbia Provincial Toxicology Centre, Vancouver, BC V5Z 4R4, CANADA; Andrew Tu, PhD, British Columbia Coroners Service, Burnaby, BC, CANADA; Michael Moss, MBBS, Interior Health Authority, Kelowna, BC V1Y 1T2, CANADA; Jatinder Baidwan, MBBS, British Columbia Coroners Service, Burnaby, BC V5H 4N2, CANADA

**Learning Overview:** After attending this presentation, attendees will: (1) appreciate the challenges faced by coroner/medical examiner systems considering the recent opioid crisis, (2) learn the differences between presumptive immunoassay and confirmatory mass spectrometry, and (3) understand the relationship (or lack thereof) between drug concentrations in the blood and urine.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the potential benefits and detriments of using presumptive screening to limit the number of autopsies performed in suspected overdose deaths.

**Background/Introduction:** The province of British Columbia, Canada, has seen an unprecedented increase in the number of opioid-related deaths in recent years. In 2017, there were 1,156 fentanyl-related deaths, which represent 81% of all illicit drug deaths in the province. The British Columbia Coroners Service stratifies decision-making on postmortem examination prior to autopsy and releases bodies in cases in which fatal drug findings are identified. In cases in which an immediate toxicological cause of death cannot be identified, decedents undergo full postmortem examination. There is a limited timeframe in which a body can be stored prior to autopsy. Toxicology results must therefore be performed within days of collection. To expedite fentanyl testing, the implementation of urine screen immunoassay has been proposed.

**Objective:** The purpose of this study is to evaluate the robustness of presumptive urine drug screening in suspected fentanyl-related deaths by establishing the relationship between fentanyl and norfentanyl in blood and urine.

**Method:** Suspected drug-related deaths that occurred within the Interior Health Region of the Province of British Columbia were included in this study if blood and urine were available for testing. One aliquot of urine was sent to clinical laboratories within the Interior Health region for presumptive testing using the SureStep™ urine fentanyl drug test (cutoff concentrations: >20ng/mL norfentanyl, >100ng/mL fentanyl). Blood and a second aliquot of urine were sent to the Provincial Toxicology Centre for complete toxicology testing by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Concentrations of fentanyl and norfentanyl in blood and urine were then compared to the presumptive results. A concentration of 3ng/mL was the minimum concentration at which fentanyl could be associated with death.

**Results:** Preliminary results from 29 cases found that 72% of presumptive urine screen results coincided with blood fentanyl results. There were five false negatives, in which the presumptive screen did not detect fentanyl but the concentration in the blood exceeded 3ng/mL, and three false positives (Se=72%; Sp=73%; PPV=81%; NPV=62%). Urine fentanyl and norfentanyl concentrations determined by LC/MS/MS in acute overdose deaths did not strongly correlate to those of blood (correlation coefficient=0.12 and 0.65 for fentanyl and norfentanyl, respectively). All presumptive screen results in which fentanyl was detected contained norfentanyl; however, 25% of those cases were below the reported cutoff. In contrast, 8% of cases in which the presumptive screen did not detect fentanyl were above the reported cutoff. Urine carfentanil concentrations ranged from 0.2ng/mL to 2.0ng/mL. No carfentanil-only cases were detected by the presumptive screen.

**Conclusion/Discussion:** In the majority of cases, urine presumptive screening coincided with blood fentanyl concentrations of at least 3ng/mL. Comparison of urine concentrations to presumptive screen results suggest that the high false negative rate is related to the absence of norfentanyl in the urine following acute death, rather than a sensitivity issue associated with the presumptive screen. False negative results carry minimal risk as they are followed up by complete toxicology testing and postmortem examination. However, false positives would result in the release of a body prior to autopsy in cases in which no cause of death has been determined. Further investigation into this study will provide valuable insight into whether the false positive rates constitute an acceptable risk.

#### Fentanyl, Urine Screening, Postmortem Toxicology

## K60 Distinguishing Cyclopropylfentanyl From the Analogs Crotonylfentanyl and Methacrylfentanyl in Two Dimensions: The Utilization of Liquid Chromatography and Mass Spectrometry

Svante Vikingsson, PhD\*, National Board of Forensic Medicine, Linköping 58758, SWEDEN; Markus Roman, BS, National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping 58758, SWEDEN; Robert Kronstrand, PhD, National Board of Forensic Medicine, Department of Forensic Toxicology, Linköping 58758, SWEDEN

**Learning Overview:** After attending this presentation, attendees will: (1) be aware of two fentanyl analogs that could potentially be mistaken for cyclopropylfentanyl, and (2) have the tools to distinguish them.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) providing modes of separation for cyclopropylfentanyl and closely related analogs, and (2) identifying potential pitfalls involving isobaric analogs in New Psychoactive Substances (NPS) identification.

**Background:** In 2017, Sweden experienced a series of 72 deaths involving the fentanyl analog cyclopropylfentanyl. Identification was complicated by the two isobaric analogs crotonyl- and methacrylfentanyl, which produced similar high-resolution Mass Spectrometry (MS) -spectra and similar retention times in the screening assay.

**Goal:** The goal of this study was to establish methodology that could distinguish the three analogs by Liquid Chromatography (LC) and/or MS to verify the presence of cyclopropylfentanyl in autopsy cases.

**Methods:** A screen for chromatographic selectivity was conducted. Five different Ultra High-Performance Liquid Chromatography (UHPLC) columns (50x2.1, <math><2\mu\text{m}</math>) including Waters® HSS T3, CSH Phenyl Hexyl, Protein BEH C4 and BEH C8 as well as Kinetex® PFP from Phenomenex, were screened using two different organic modifiers as B-phase (either acetonitrile or methanol with 0.05% formic acid) and two different A-phases (0.05% formic acid in either water or 10mM ammonium formate) using a generic gradient from 5%–65% organic modifier over 8min at 0.7ml/min. A 2min gradient was optimized based on the results of the screen.

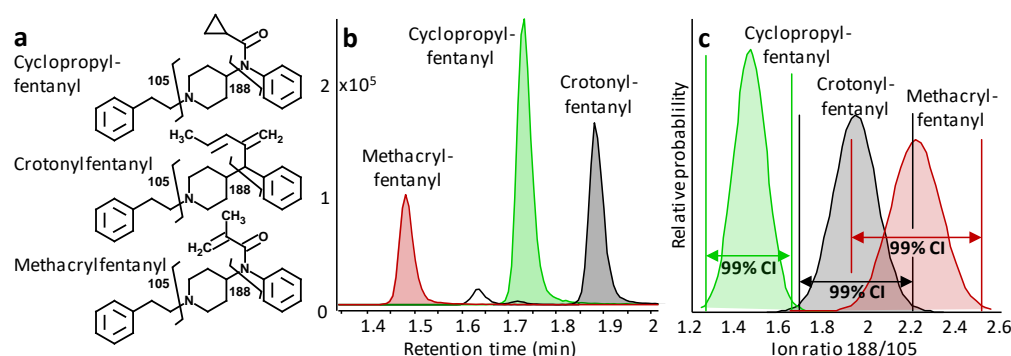
As fragmentation of the analogs was very similar (major fragments in Figure 1a), ion ratios of major fragments were investigated to distinguish the analogs. Reference ion ratios were established as the average of ten replicates analyzed by Multiple Reaction Monitoring (MRM) on an AB SCIEX® API 4500 LC/MS/MS. The separation was performed using a Waters® BEH Phenyl column (50x2.1mm, 1.7 $\mu\text{m}$ ) with 0.05% formic acid in 10mM ammonium formate and methanol as mobile phases.

The stability of ion ratios over time was investigated by analyzing data from 68 cyclopropylfentanyl autopsy cases analyzed over seven months. The standard deviation and the 99% confidence interval ( $\pm 2.6$  SD) were calculated. As no case data was available for crotonyl- and methacrylfentanyl, the same Relative Standard Deviation (RSD) was assumed.

**Results:** The best separation was obtained on a Waters® Protein BEH C4 column using 0.05 formic acid in 10mM ammonium formate and 0.05% formic acid in methanol as mobile phases. Separation with an optimized 2min gradient from 15-30% methanol is shown in Figure 1b.

Average ion ratios (m/z 188/105) for cyclopropyl-, crotonyl-, and methacrylfentanyl were 1.46, 1.94 and 2.21, respectively. The RSD for the cyclopropyl ion ratio was 5.1%. Probability profiles were plotted in Figure 1c including the 99% confidence interval ( $\pm 2.6$  SD). Cyclopropylfentanyl could be distinguished from the other analogs while an overlap existed between crotonyl- and methacrylfentanyl.

Analogues with closely related isobaric analogs is a growing concern given the increasing number of NPS, especially as laboratories may not be aware of potential analogs at the time of method validation. By utilizing selective chromatographies and carefully selecting acceptance criteria for ion ratios, the confidence in NPS identification can be increased.



**Figure 1** – Structures of the fentanyl analogs (a), optimized chromatography (b), and Confidence Intervals (CI) of m/z 188/105 ion ratios (c).



**Conclusion:** This study illustrates that even though cyclopropyl-, crotonyl-, and methacrylfentanyl have the same nominal mass and are structurally similar, it was possible to distinguish them by their retention time on a BEH C4 column.

It was also possible to distinguish cyclopropylfentanyl from crotonyl- and methacrylfentanyl by the difference in their respective m/z 188/105 ion ratios. However, crotonyl- and methacrylfentanyl could not be distinguished from each other using only ion ratios. These approaches could potentially be used to distinguish other NPS drugs from isobaric analogs and provide more reliable identifications in toxicological casework.

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### **Cyclopropylfentanyl, Crotonylfentanyl, Methacrylfentanyl**



## K61 Method Optimization for the Derivatization of 35 Drugs Commonly Reported in Driving Under the Influence of Drugs (DUID) Cases

Lexus R. Rutter, BS\*, Arcadia University, Glenside, PA 19038; Jody Searfoss, BA, Glenside, PA 19038-3215; Kristina Fanning, MS, United Chemical Technologies, Bristol, PA 19007; Danielle A. Mackowsky, MS, United Chemical Technologies, Bristol, PA 19007; Karen S. Scott, PhD, Arcadia University, Glenside, PA 19038

**Learning Overview:** After attending this presentation, attendees will have an increased understanding of which derivatizing agent and technique are best suited for the analysis of a variety of common drugs of differing chemistries.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing forensic laboratories with a derivatization and Gas Chromatography/Mass Spectrometry (GC/MS) method for Tier 1 drugs and metabolites associated with DUID cases.

DUID encompasses prescription, over-the-counter, and controlled substances that cause impairment of one's judgment and motor skills. A series of recommendations has been published that describes two tiers of drugs that are commonly seen in DUID cases.<sup>1</sup> Screening and confirmatory cutoff limits have been recommended for the Tier 1 drugs to create better consistency across laboratories.<sup>1</sup>

Typical casework for many forensic toxicology laboratories for DUIDs includes blood and urine screenings. There is a move internationally from GC to Liquid Chromatography (LC) techniques, but many laboratories still lack instrumentation such as a triple quadrupole LC/MS/MS. For most laboratories, derivatization is therefore a critical step for certain drugs to be suitable for GC/MS analysis.

The Tier 1 drugs consist of four classes of drugs. The Central Nervous System (CNS) stimulants include methamphetamine, amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), 3, 4-methylenedioxyamphetamine (MDA), cocaine, benzoylecgonine, and cocaethylene. The CNS depressants consist of carisoprodol, meprobamate, zolpidem, alprazolam,  $\alpha$ -hydroxyalprazolam, clonazepam, 7-aminoclonazepam, lorazepam, diazepam, nordiazepam, oxazepam, and temazepam. Codeine, 6-acetylmorphine, buprenorphine, norbuprenorphine, fentanyl, hydrocodone, hydromorphone, methadone, morphine, oxycodone, oxymorphone, tramadol, and o-desmethyltramadol make up the narcotic analgesic category. The cannabis category includes Tetrahydrocannabinol (THC), carboxy-THC, and 11-hydroxy-THC. Differences in the functional groups associated with these drugs often require different derivatizing reagents such as silylation, acylation, and alkylation reagents. This research aims to determine a single GC/MS method for the analysis of all 35 recommended Tier 1 drugs and metabolites.

A test mix was prepared by adding 1mL of a 10 $\mu$ g/mL solution for each of the 35 drugs in acetonitrile. Seven derivatizing reagents, Pentafluoropropionic Acid Anhydride (PFPA), Trifluoroacetic Acid Anhydride (TFAA), Heptafluorobutyric Anhydride (HFAA), N, O-Bis (Trimethylsilyl)Trifluoroacetamide with 1% Trimethylchlorosilane (BSTFA with 1% TMCS), N-Methyl-N-Trimethylsilylfluoroacetamide (MSTFA), N-methyl-N-(tert-butyltrimethylsilyl) trifluoroacetamide with 1% Tert-Butyltrimethylchlorosilane (MTBSTFA with 1% TBDMCS), and Trimethylanilinium Hydroxide (TMAH) were assessed. Triplicate samples of 100  $\mu$ L of the test mix were derivatized for each reagent changing the temperature and time of incubation followed by GC/MS analysis.

Separation of all 35 drugs was achieved on an Agilent® 6890 GC 5975 MS using an Agilent® DB-5MS Ultra Inert (30m x 0.25mm x 25 $\mu$ m) capillary column with a temperature program starting at 60°C and ending at 325°C. MSTFA and PFPA yielded the most consistent results across the range of chemistries of the drugs and it was possible to obtain sharp, reproducible baseline-resolved peaks at concentrations equal to the cutoff values stated in the DUID recommendations for the 35 Tier 1 drugs and metabolites.<sup>1</sup>

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### Derivatization, GC/MS Analysis, DUID



### K62 A Case Report: Driving Under the Influence (DUI) of Flubromazepam

Erin L. Karschner, PhD\*, Armed Forces Medical Examiner System, Dover Air Force Base, DE 19902; George F. Jackson, PhD, Forensic Toxicology, Burlington Township, NJ 08016; Jeff Walterscheid, PhD, Armed Forces Medical Examiner System, Division of Forensic Toxicology, Dover Air Force Base, DE 19902

**Learning Overview:** After attending this presentation, attendees will be aware of the impairment effects demonstrated by an individual DUI of a benzodiazepine Novel Psychoactive Substance (NPS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing quantitative data from a single drug intoxication case that may assist toxicologists in future casework interpretation.

Benzodiazepines are a commonly prescribed class of drugs used to treat anxiety, seizures, and sleep disorders. Several decades ago, investigators synthesized a number of benzodiazepines as potential new treatment options. More recently, these substances have reemerged for sale on the internet as legal highs. Flubromazepam was first developed and patented in the 1960s and reappeared on the internet marketplace in 2012. Currently, few reports are available from single drug intoxications involving flubromazepam.

**Case History:** Police were notified of a vehicle driving erratically and swerving between lanes. The 23-year-old male driver was stopped after his vehicle hit a curb. The individual was unable to hold a steady conversation and had difficulty comprehending the questions asked by the officer. The officer administered Standardized Field Sobriety Tests (SFSTs) and observed indicators for lack of smooth pursuit in both eyes on the Horizontal Gaze Nystagmus (HGN) test and six clues on the Walk And Turn (WAT), including inability to maintain balance during the instruction phase, started too soon, missed heel-to-toe, raised arms, incorrect number of steps, and improper turn. No additional SFSTs were conducted due to the observations made during the HGN and WAT. An officer initiated a search of the vehicle and discovered empty prescription bottles of sertraline and oxycodone, a small bag of blue pills, a small bag containing traces of a white powder, and white powder located in the compartment above the gearshift. The individual threw himself to the ground while he was being detained and was injured. At that time, he was transported to a medical facility where blood was drawn and submitted to the Armed Forces Medical Examiner System Division of Forensic Toxicology.

Routine toxicological analyses included ethanol and additional volatiles, drugs of abuse immunoassay, and an alkaline-extractable drug screen. Blood volatiles were analyzed by headspace Gas Chromatography/Flame Ionization Detector (GC/FID). Immunoassays were performed for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, opioids, phencyclidine, and sympathomimetic amines. Alkaline drugs were extracted with a mixed-mode solid phase extraction procedure and analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) in full-scan mode. Identification criteria included retention time within  $\pm 2\%$  of the analyte in the control standard and a full-scan mass spectrum matching a reference spectrum with at least 70% confidence. Subsequent confirmation was conducted with a validated method for liquid-liquid extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis. Two Multiple Reaction Monitorings (MRMs) were monitored for all analytes and deuterated internal standards. Qualitative identification was dependent on meeting MRM ratio, peak shape, and retention time criteria. Quantitative criteria included control bias within 20% of target and calibration curves with  $r^2 \geq 0.99$ .

The blood was negative for ethanol but was presumptively positive for benzodiazepines by immunoassay. An alkaline drug screen narrowed the benzodiazepine presumptive positive to flubromazepam with a 76% spectral library match. Confirmatory analysis revealed that the blood contained flubromazepam at 546ng/mL.

This study presents a DUID case where flubromazepam was the only substance identified in the blood. In addition to the erratic driving behavior, the individual displayed two clues on the HGN and six of eight clues on the WAT. Video evidence also showed the individual stumbling and hitting walls as he left his room 20min prior to the DUI incident. Lack of smooth pursuit, impaired divided attention, and other central nervous system depressant outcomes observed in this individual were similar to effects elicited by traditional benzodiazepines.

#### DUID, Flubromazepam, NPS



### K63 Toxicological Findings in Driving Under the Influence Cases in Northeast Ohio: A Six-Year Study

Amanda J. Jenkins, PhD\*, University of Massachusetts Memorial Medical Center, Worcester, MA 01605; Kimberly Yacoub, MS, Westlake, OH 44145; Douglas E. Rohde, MS, Lake County Crime Laboratory, Painesville, OH 44077

**Learning Overview:** After attending this presentation, attendees will have a better understanding of drug prevalence in a recent Driving Under the Influence/Driving Under the Influence of Drugs (DUI/DUID) population.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data on recent drugged drivers in the United States.

**Background:** There are more than six million motor vehicle accidents in the United States annually. Drug testing of operators of motor vehicles has become a routine occurrence, especially if serious injuries or fatalities occur. Knowledge of the prevalence of drugs in these individuals will provide evidence to assist policy makers in the public health effort to improve roadway safety.

**Objective:** The objective of this study was to review all driving cases submitted to the Lake County Crime Laboratory for toxicology testing by law enforcement during the period January 1, 2012, to December 31, 2017, in Lake and Ashtabula Counties, Ohio.

**Methods:** Blood and/or urine were submitted for each case and a standard protocol utilized to determine the tests to be conducted. Testing for volatiles by headspace gas chromatography in blood and urine was initially performed. If the ethanol concentration was below the per se level (per se=blood 0.08; urine 0.11 g%), the specimen was assayed by enzyme-linked immunosorbent assay screen for amphetamine, barbiturates, benzodiazepines, cannabinoids, carisoprodol, cocaine/metabolites, fentanyl, methadone, methamphetamine, opiates, oxycodone, phencyclidine, tramadol, tricyclic antidepressants, and zolpidem. Screening positive results were confirmed by Gas Chromatography/Mass Spectrometry (GC/MS). A general drug screen by solid phase extraction followed by GC/MS for alkaline, neutral, and acidic drugs was also performed based upon case history. Additional confirmatory testing was performed as indicated by the case narrative, further investigation, or law enforcement request.

**Results:** A total of 845 cases were identified. Blood and urine were submitted in 25% of the cases with 46% blood-only and 29% urine-only submissions. Only confirmed drug testing results are presented. No drugs, including ethanol, were detected in 30 cases (3%). The most common drug detected was ethanol ( $n=442$ ; 52%, range; mean and median (g%)- blood 0.010–0.410; 177, 174; urine 0.012–0.474; 210, 215) followed by opioids (36%), benzodiazepines (22%), and marijuana (21%). Other prevalent drugs included cocaine/metabolites, sympathomimetic amines, and carisoprodol/meprobamate. More than one drug were identified in 319 cases (37% of the total number of cases) with 93 cases (11% total) containing more than three compounds (62% of these occurred 2015–2016). Barbiturates and phencyclidine accounted for less than 3% of the positive cases. The highest number of individual drugs associated with a single case was 12. Codeine/morphine was the most prevalent opioid combination and alprazolam, clonazepam/7-aminoclonazepam, and diazepam/metabolites were prevalent for the benzodiazepines. Trends observed included the proportion of cases positive for ethanol decreased over the study period, from 66% of all cases in 2012 to 37% in 2017. The proportion of cases positive for opioids increased from 2012 (18%) to a high of 57% in 2016.

**Conclusion:** A six-year evaluation of potential drugged driving cases demonstrated that at least one drug was detected in more than 95% of cases. Furthermore, a noteworthy number of cases reflected poly drug use.

#### Driving Under the Influence, Impaired Driving, Toxicology





### K64 Inhalant Abuse: Cases Analyzed for Toluene Over a Three-Year Period

*Justine M. Titko, MSFS\*, NMS Labs, Willow Grove, PA 19090; Lee M. Blum, PhD, NMS Labs, Willow Grove, PA 19090; Dennis J. Miller, DeKalb County Coroner's Office, Sycamore, IL 60178; Ayako Chan-Hosokawa, MS, NMS Labs, Willow Grove, PA 19090*

**Learning Overview:** After attending this presentation, attendees will understand the toluene blood concentrations in suspected overdose death cases and the benefits of pursuing an unknown chromatographic peak observed during the toxicological analysis of a postmortem specimen.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing statistical information associated with toluene findings in postmortem cases and provide insight into a specific case of toluene abuse.

This presentation focuses on a review of 56 cases over a three-year period in which toluene was detected via dual column headspace Gas Chromatography with Flame Ionization Detection (GC/FID) (RL=0.3mcg/mL). Of these cases, 52 provided information in the case history about the decedent's gender; 85% ( $n=44$ ) were males, while 15% ( $n=8$ ) were females. The 56 cases were then subdivided into three subcategories based on case history: Huffing or Solvent Abuse Suspected Deaths ( $n=11$ , 20%); Other Causes of Death ( $n=5$ , 9%); and No Case History Provided ( $n=40$ , 71%).

To assess toluene concentrations in postmortem cases, the 11 postmortem cases in which huffing or solvent abuse was suspected were examined. The mean ( $\pm$ SD) blood concentration of toluene in this subset of cases ( $n=11$ ) was  $16.8 \pm 36.2$ mcg/mL (median=3.1mcg/mL; range: 0.63mcg/mL–130mcg/mL). Of the 11 cases, 82% were male and 18% were female. The ages provided for 73% of the cases averaged 45.5 years old (range; 22–62 years old) with the median and mode both at 50 years old. Additional findings observed in these cases included other volatile organic compounds, ethanol, acetone, isopropanol, methanol, prescribed medications, and drugs of abuse.

There was one specific case that illustrated the importance of pursuing an unidentified chromatographic peak during a toxicological examination. The case involved a 63-year-old male found unresponsive at his residence after his mother called the police to perform a welfare check on him. He was found in the basement with a plastic bag over his head and a jar nearby containing a clear liquid that smelled like paint thinner. The decedent had a history of huffing paint thinners for approximately 13 years, and toluene is a known ingredient in some types of paint thinners. Autopsy findings included, but were not limited to, a decomposed body with autolysis of organs, pulmonary edema and congestion, and prostatic hypertrophy.

A routine postmortem toxicology drug panel indicated non-lethal amounts of ethanol and caffeine in cavity blood and a non-lethal amount of ethanol in vitreous fluid. However, the investigation of an unknown substance in an analysis for alkane constituents of solvents resulted in supplemental testing in the cavity blood. First, the blood specimen was analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) using full-scan mode that identified toluene and isovaleraldehyde. Then, the presence of these findings was quantitated by dual column headspace GC/FID. The toluene measured, 2.53mcg/mL, was likely an underreported value because of the volatility of the substance and the handling of the specimen multiple times prior to the analysis for toluene. The cause of death was determined to be toluene intoxication.

Toluene has been abused through the years (e.g., glue sniffing and huffing) because of its ease of accessibility and low cost. Specimens from individuals suspected of inhalant solvent abuse require specialized handling and testing due to the volatile nature of these compounds. Such considerations need to be considered when investigating these types of cases.

#### Toluene, Inhalant, Overdose



### **K65 The Detection and Quantification of Amphetamine and Norephedrine in Rat Brain, Heart, and Liver Tissues at Different Stages of Decomposition After Internment**

*Christina Rose Mendralla, BS\*, Arcadia University, Glenside, PA 19038; Jolene Bierly, MSFS, NMS Labs, Willow Grove, PA 19090; Heather L. Harris, JD, Glenside, PA 19038; Karen S. Scott, PhD, Arcadia University, Glenside, PA 19038*

**Learning Overview:** After attending this presentation, attendees will be aware of the possible relationship between antemortem amphetamine dose and the concentration of amphetamine and its metabolite norephedrine in buried remains at various stages of decomposition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing information regarding the effect of decomposition on the identification and quantification of amphetamine and norephedrine in postmortem tissue samples. This will aid death investigators in determining a possible drug contribution to cause of death when amphetamine is present.

In forensic toxicology casework, the primary matrices include biological fluids, such as blood and urine. However, some forensic casework includes the testing of decomposed postmortem tissue samples. Forensic toxicologists, therefore, need to know if decomposition has an effect on the identification and quantification of a drug because any analysis completed by the toxicologist assists the forensic pathologist in determining the impact of the drug(s) on the cause of death. This involves the understanding of taphonomy and its effects. Due to this intersection of toxicology and taphonomy, this research aims to determine the relationship between antemortem amphetamine dose, the concentration of amphetamine, its metabolite norephedrine, and the stage of decomposition of buried remains.

This relationship is important for amphetamine as it is a drug that is seen in fatal drug cases, so a possible relationship between decomposition and the concentration detected could have an impact on its interpretation in postmortem samples. To determine this relationship, 17 male Long Evans rats were dosed with amphetamine at concentrations of 10mg/kg, 6mg/kg, 2mg/kg, 1mg/kg, 0.6mg/kg, and 0.2mg/kg once a day for ten days. The rats dosed at the 10mg/kg, 6mg/kg, and 2mg/kg concentrations were euthanized with CO<sub>2</sub> ten days after the final injection while the other doses were euthanized with CO<sub>2</sub> immediately following the final dose. The rats were then interred in the New Jersey Pine Barrens, then later exhumed at different stages of decomposition in accordance with the Megyesi method. Following exhumation, liver, heart, and brain tissue samples were collected during dissection, but when decomposition was more advanced, samples were collected from the general location at which the organ would have been found. The samples were stored at -20°C until analysis. During analysis, the samples were tested for amphetamine and its metabolite norephedrine.

The samples were prepared by homogenizing the tissues with saline using a Biotage® Bead Ruptor 24 (1:1 for brain and liver, 2:1 for heart) and performing a protein precipitation with acetonitrile followed by a Liquid-Liquid Extraction (LLE) for amphetamine and norephedrine. The LLE method involved an initial organic extraction using dichloromethane/ethyl-acetate/isopropyl alcohol (3:1:1), a back extraction using a 1M phosphate buffer at pH 2.5, and a final organic extraction using dichloromethane/isopropyl alcohol/ammonium hydroxide (78:20:2). The analytes were identified and quantified using a Perkin Elmer Clarus® SQ 8T Gas Chromatograph/Mass Spectrometer (GC/MS) in Selected Ion Monitoring (SIM) mode with amphetamine-D11 (m/z 194) and norephedrine-D3 (m/z 283) as the internal standards. The SIM ions for amphetamine are 190, 118, and 91; the SIM ions for norephedrine are 280, 190, and 91. The column used in this research is an Agilent® DB-5ms Ultra column (30m, 0.25mm ID, 0.25µm df). This method was validated using the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for method validation.

The analysis of the postmortem tissue samples showed that amphetamine and norephedrine can be detected in soft tissue through the different stages of decomposition. A dose-related concentration was seen within the two groups of rats.

#### **Amphetamine, Buried Remains, Postmortem Tissue**



## K66 The Stability of Antidepressants and Antipsychotics in Dried Blood Spots (DBS) in Postmortem Cases

Matteo Moretti, MD\*, University of Pavia, Section of Legal Medicine and Forensic Sciences, Pavia 27100, ITALY; Francesca Freni, MSc, Arma Di Taggia, ITALY; Silvia D. Visona, MD, University of Pavia, Pavia 21100, ITALY; Claudia Vignali, University of Pavia, Pavia, ITALY; Angelo Groppi, University of Pavia, Pavia 27100, ITALY; Antonio M.M. Osculati, MD, University of Pavia, Pavia 27100, ITALY; Luca Morini, University of Pavia, Pavia 27100, ITALY

**Learning Overview:** After attending this presentation, attendees will understand the reliability of the analysis of blood collected in postmortem cases and dried on a paper substrate (i.e., DBS), particularly for the detection and quantification of antidepressants and antipsychotics.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing new information about the use of DBS in forensic toxicology and the possibility of employing this technique as a good, simple, reliable, and complementary method of sample storage during autopsy.

**Background and Goals:** The analysis of blood collected and dried on a paper substrate (DBS) represents an alternative blood sample collection that is currently routinely used in neonatal screening. The analysis of DBS provides several advantages: (1) it requires a small volume of blood; (2) it needs easier sample handling and simple storage for a longer time periods at room temperatures; and (3) the dried blood matrix could limit or even avoid the postmortem changes, such as putrefaction or autolysis. The goals of this study were: (1) to develop a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for the determination of 22 antidepressants and 19 antipsychotics in DBSs; (2) to establish the diagnostic reliability of DBS with the routine blood analyses of these substances; and (3) to prove the stability of these analytes on DBS within a three-month period of storage.

**Method:** Aliquots of blood were pipetted on a five-spot filter paper (85µL of blood for each spot) in triplicate (so that further testing is possible). The cards were allowed to dry overnight in the dark at room temperature. For each analysis, a whole bloodstain was cut out and placed in a plastic tube containing 1mL phosphate buffer at pH 6 and deuterated internal standards, extracted using Solid Phase Extraction (SPE) cartridges, and injected into the LC/MS/MS system. The analytes were separated through a reverse phase chromatography on a C18 column and detected on a triple quad operating in Multiple Reaction Monitoring mode and positive polarization. A calibration curve was prepared in the range of Limit of Quantitation (LOQ) 500ng/mL.

Validation parameters (linearity, selectivity, specificity, accuracy, imprecision, recovery, matrix effects, and carry over) were tested. The procedure was applied to 60 authentic postmortem cases. For each case, 15 DBS were collected at T0. Four DBS were analyzed in triplicate within four consecutive weeks. The last samples were analyzed after three months.

**Results:** The analytical procedure is simple, sensitive, and specific. Limits of Detection (LODs) were detected in a range of 0.1ng/mL(g) to 4.1ng/mL(g) for antidepressants, and 0.1ng/mL to 2.7ng/mL for antipsychotics. LOQs varied from 0.3ng/ml to 13.6ng/ml for antidepressants and 0.3ng/ml to 9.1ng/mL for antipsychotics. Validation parameters fulfilled all the acceptance criteria. Eighteen different molecules were detected and quantitated in 22 out of 60 cases.

Four cases were positive for citalopram (42ng/ml–408ng/mL) and quetiapine (84 ng/ml –2309ng/mL), 3 for desvenlafaxine (479ng/ml–686ng/mL) and venlafaxine (25ng/ml–362ng/mL), 2 for paliperidone (17ng/ml–56ng/mL) and trazodone (91ng/ml–162ng/mL), while amisulpride (527ng/mL), clotiapine (25ng/mL), dibenzepine (483ng/mL), dixyrazine (126ng/mL), fluoxetine (56ng/mL), fluvoxamine (4,300ng/mL), haloperidol (20ng/mL), maprotiline (340ng/mL), mirtazapine (134ng/mL), and N-desmethylmirtazapine (112ng/mL), paroxetine (671ng/mL) and pimozone (130ng/mL) were found only once.

The concentrations on DBS stored at room temperature were in good agreement with the ones obtained on blood samples analyzed with routine methods (<30% CV, both at T0 and after three months).

The degradation percentage for most of the substances was lower than 20% within the three-month period. Citalopram, trimipramine, and paliperidone were stable for the first month, while a degradation higher than 50% was observed after three-month storage. Quetiapine was found to be stable into two samples, while a significant degradation was noticed in two other cases.

**Conclusions:** A method for the detection and quantitation of antidepressants and antipsychotics in DBS was successfully developed and validated. Preliminary results on 22 authentic positive postmortem cases suggested that DBS can be used as routine sample storage during autopsy. More cases will be analyzed to support the preliminary outcomes of this study.

### Dried Blood Spot, Antidepressants, Antipsychotics



### K67 A Rare Case Report on Sedative-Hypnotic Drug Screening in Homicidal Carbon Monoxide (CO) Poisoning

Linchuan Liao\*, Sichuan University, Chengdu, Sichuan 610041, CHINA

**Learning Overview:** After attending this presentation, attendees will understand a rare case of deliberate homicide via CO poisoning.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the importance of sedative-hypnotic drug screening in the case of CO poisoning.

CO has been considered the number one “Silent Killer.” In forensic practice, deaths due to CO poisoning are usually suicidal or accidental; homicidal CO poisoning is very rare. The current report presented an ingenious crime of homicidal CO poisoning, which is expected to emphasize the importance of sedative-hypnotic drug screening in the case of CO poisoning.

**Case:** Two men (Decedent A and B) in their fifties were found dead in a car parked in an open parking area in winter. Decedent A was lying on the reclining driver’s seat and Decedent B was lying prostrate on the reclining passenger seat. All doors and windows were locked. The ignition key was in the “ON” position, but the engine was not running. There was neither a suicide note in the car nor at the home of the victims. In the meantime, it was discovered that about \$75,000 cash carried by the decedents was missing. Autopsies were subsequently conducted as requested by both decedents’ wives.

**Autopsy Findings:** There were no notable injuries on the surface of the two bodies. The sclera and palpebral conjunctivae showed some petechiae in both bodies. Both of the decedents exhibited cherry-pink lividity, which did not fade upon finger pressure. Internal examination revealed that the musculature and internal organs were bright red in color. Sporadic petechiae were present on the surface of the heart, liver, lungs, and spleen.

**Toxicological Analyses:** Cardiac blood samples from both decedents were obtained at the autopsy for toxicological analyses of carbonyl hemoglobin (COHb), alcohol, and benzodiazepines. Blood COHb saturation of Decedent A and Decedent B determined by visible spectrophotometry were found to be 70.5% and 69.5%, respectively. Concentrations of alcohol in the blood samples of Decedent A and Decedent B determined by Headspace/Gas Chromatography (HS/GC) method were found to be at 0.20mg/ml and 1.02mg/ml, respectively. For analyses of benzodiazepines, blood samples were treated by liquid-liquid extraction with ethyl acetate, followed by identification and quantification analysis using Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS). Estazolam was detected in the blood of both decedents at 0.31µg/ml and 0.10µg/ml in Decedent A and Decedent B, respectively.

**Discussion:** Since fatalities can take place when the CO saturation of blood rises above 40%, it seemed reasonable to judge that the cause of the deaths was CO poisoning. However, there were two questionable points in this case: (1) \$75,000 cash was missing; (2) estazolam was detected in both decedents (both decedents had no medical history of estazolam). Decedent A’s wife told the police that her husband had an appointment with his business partner C and never came back after that. After repeated interrogation, C eventually confessed that he put estazolam in the decedents’ drinks and drove the two sleeping persons to the suburb; then he locked them in his car and poisoned them with a prepared CO bottle, followed by moving the bodies back to Decedent A’s car, reclining the seats and turning on the ignition key, making it look like an accident. Although homicidal CO poisoning is very rare in forensic practice, this current case emphasizes the importance of additional screening for sedative-hypnotic drugs in the case of CO poisoning.

#### Homicidal CO Poisoning, Toxicological Analysis, Case Report



### K68 Medicinal Drug Suicide: A Six-Year Forensic Survey

Asit Kumar Sikary, MD\*, ESIC Medical College & Hospital, Faridabad, Haryana 121001, INDIA; Anu Sasidharan, MD, Amrita School of Medicine, Department of Forensic Medicine, Kochi 682026, INDIA; V.V. Pillay, MD, Amrita Institute of Medical Sciences, In-charge, Poison Control Centre, Kochi 682026, INDIA; Chittaranjan Andrade, MD, National Institute of Mental Health & Neuroscience, Department of Psychopharmacology, Bangalore 560029, INDIA

**Learning Overview:** After attending this presentation, attendees will better understand the increasing trends of medicinal drug abuse for suicidal purposes in a metro city of India due to the easy availability of over-the-counter drugs and prescriptions for others.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by making attendees aware of the increasing problem of suicide by medicinal drugs that are prone to being missed if detection is not suspected.

Medicinal drug abuse has increased in recent years across the United States, Europe, and Australia and has acquired the top position along with other drugs of abuse. The most commonly abused medicinal drugs are opioids, followed by tranquilizers and psychotropic drugs. Medicinal drugs are easily available at home, are easy to obtain from a pharmacist on other people's prescriptions or even without a prescription (over the counter), are not illegal or expensive, are associated with less social stigma, are easily available over the internet, and have fewer side effects than street drugs.

The abuse is increasing in India, too, as reported by the United Nations Office on Drugs and Crime. According to the report, pharmaceutical drugs can be procured easily over the counter without any prescription. These are mainly synthetic opioids, tranquilizers, sedatives, and stimulants. These drugs are cheap compared to usual abusive drugs and are within the reach of the lower class. The National Crime Record Bureau of Government of India says that fatal medicinal drug abuses are mainly accidental overdoses used for various ailments and are procured over the counter from medical stores without any prescription.

In this study, cases of suicide with medicinal drugs from the South Delhi region of India were analyzed. Data on suicide by medicinal drugs were retrospectively analyzed. Drugs recovered from the scene of suicides were noted for their constituents and strength. Doses taken for suicide were estimated by considering the number of empty strips/vials of the drug that were used at the time of suicide. Relatives and friends of the deceased were interviewed at the time of the autopsy regarding the occupation of the victim, the source of the drug, and any history of abuse of that particular drug or any other drugs. The presence of the drug in the blood of these cases was confirmed through the reports from the Forensic Science Laboratory and from the departmental laboratory.

During this period of study, most of the suicides were due to hanging (~75%), followed by poisoning (~15%). Other causes of suicide were drowning, fall from height, and gunshot. There were 338 cases (201 males and 137 females) of suicidal poisoning; most of them were with pesticides used in agriculture (35%) and household chemicals (18%). Twenty males and seven females (8.0%) had committed suicide with medicinal drugs. The age range of males was 21-34 years while the age range of females was 22-32 years. Most of the males (45%) were students and the rest were mainly manual unskilled workers or unemployed. Among the females, most were housewives and students (43% each). The students had good knowledge of the various drugs available over the counter. Four victims were practicing doctors having access to various drug preparations. Out of 27 cases, 11 victims had consumed a drug having a combination of dextropropoxyphene (opioid analgesic) and dicyclomine (anticholinergic), mainly used as antispasmodic. Four victims consumed two drugs of different constituents, mainly combining alprazolam with others. The rest of the victims used drugs with a single constituent only. In three cases, drug constituents were not known. In 13 cases (48%), the drugs were obtained directly over the counter from the medical store without any prescription. In six cases, the drugs were prescribed by the treating physician for the victim or for a relative of the victim for various ailments. These legitimate prescriptions were mainly antispasmodics; one prescription was an antiepileptic and another prescription was an antidepressant. In three cases, the type of drug consumed was unknown, as the description of the content on the strip was not legible. How the victim acquired the drugs was not known. As per history, however, the drugs were not stored at home.

This study draws attention to the suicidal abuse of various medicinal drugs in an Indian metro city, which are easily available over the counter without any prescription or, with a prescription meant for oneself or for others. This method of suicide is easily preventable with the proper vigilance of prescribing doctors, dispensing chemists, and family members regarding suicidal abuse of various medicinal drugs.

#### Pharmaceutical Drugs, Over-the-Counter Drugs, Drug Overdose



## K69 Infant and Toddler Deaths Associated With Potent Opioid Exposure

*Robert Hargrove, BS\*, North Carolina Office of the Chief Medical Examiner, Raleigh, NC; Sandra C. Bishop-Freeman, PhD\*, North Carolina Office of the Chief Medical Examiner, Raleigh, NC 27607; Ruth E. Winecker, PhD, RTI International, Raleigh, NC*

**Learning Overview:** After attending this presentation, attendees will understand the dangers of potent medications as they relate to infants, toddlers, and young children. Case studies from the state of North Carolina will demonstrate what substances can harm an infant or child with environmental exposure—one swallow or pill.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by increasing awareness of the impact of the opioid epidemic on infants and toddlers and by educating forensic investigators, pathologists, and postmortem toxicologists.

While most ingestions of household products are benign, there are a handful of substances in which one pill or a small dose of medication will be sufficient to cause death in a small child, toddler, or infant. This concept is even more alarming with the modern-day opioid epidemic, which was initially fueled by generous prescribing habits and later gave rise to potent illicit fentanyl analogues. Unlike a typical adult ingestion for purposes of self-harm or pleasure, pediatric poisonings are usually the result of curiosity, exploration, a decreased or lack of sense of danger, or imitation of adults and older sibling behavior.

Due to body composition and size of an infant or toddler compared to an adult, it's not surprising to see that what may not cause death in an adult can easily cause a small child to succumb. Ingestions of a single dose of prescription medication can produce elevated concentrations in postmortem blood that may be confused with an intake of multiple doses. Without careful consideration and thorough investigations, these rulings may be considered intentional homicidal poisonings instead of accidental poisonings. Cases involving oxycodone, liquid methadone, and fentanyl will be discussed in detail. The popularity of highly potent opioids makes modern-day illicit substances far more dangerous to children. North Carolina has seen two cases of fentanyl analogue poisoning that have been certified as means of poisoning with undetermined manner by the pathologists.

Age/Sex	Weight	Toxicology	Select History
3 years/F	15 kg	Vena Cava Blood - 0.44mg/L Methadone Liver- 1.9mg/kg	A red substance was left in a food container in reach of the child, later confirmed to be methadone prescribed to the mother.
9 months/F	8.7 kg	Central Blood- 6-AM Present, 6.3ng/mL Acetyl Fentanyl, 23ng/mL Fentanyl	Infant was seen alive at 9:30 a.m. and found unresponsive at 2:25 p.m. A small piece of folded paper was recovered from gastric contents and was positive for 6-Acetylmorphine (6-AM) acetyl fentanyl, fentanyl, and morphine.

Multiple opioid case studies in toddlers and infants will be explained in detail to show incidents in which, after a thorough investigation, it was concluded that a child was exposed to a small amount of illicit substance or pharmaceutical preparation.

### Pediatric Poisoning Deaths, Postmortem, Forensic Toxicology

## K70 A Comparison of Cannabinoid Concentrations in Central and Peripheral Postmortem Blood Samples

Daniel S. Isenschmid, PhD\*, NMS Labs, Willow Grove, PA 19090; William H. Anderson, PhD, NMS Labs, Willow Grove, PA 19090; Barry K. Logan, PhD, NMS Labs/ Center for Forensic Science Research & Education, Willow Grove, PA 19090

**Learning Overview:** The goal of this presentation is to provide toxicological evidence that demonstrates the limitations of interpreting postmortem cannabinoid concentrations in blood.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing data demonstrating the variability of cannabinoid concentrations in blood collected from central and peripheral sites in medicolegal death cases.

**Introduction:** Variable concentrations of Delta-9-Tetrahydrocannabinol (THC) and its metabolites, 11-OH-Tetrahydrocannabinol (OH-THC) and Delta-9-Carboxy-Tetrahydrocannabinol (THCC), have been reported when comparing postmortem specimens collected at different times during the postmortem interval and from different sites in the body. This is the first study to compare central and peripheral blood cannabinoid concentrations in blood collected at the same time in a larger number of cases.

**Methods:** Central blood (identified as heart, chest, inferior vena cava, or subclavian) and peripheral blood (identified as femoral or peripheral) were collected during routine postmortem examinations. Peripheral blood was initially screened for cannabinoids using Enzyme-Linked Immuno-Sorbent Assay (ELISA) (cutoff concentration, 10ng/mL). Presumptive positive cases were subjected to confirmatory testing by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) for both peripheral and central blood samples. Additionally, antemortem samples were tested, when available. LC/MS/MS cut-off concentrations and Upper Limits Of Linearity (ULOL) were THC (0.5, 50ng/mL), OH-THC (1.0, 100ng/mL) and THCC (5.0, 500ng/mL). The ratio of the peripheral to central concentration of each analyte was determined for those analytes within the linear range of the assay. Additionally, a linear regression analysis between peripheral and central cannabinoid concentrations was performed. Analytes quantitating below the cutoff and greater than the ULOL were not included in the calculations.

**Results:** A total of 62, 65, and 39 pairs of blood samples were compared for THC, THCC, and OH-THC, respectively. As expected, the highest mean (3.16) and median (1.57) peripheral to heart blood ratio was for THC although the range was large (0.30–30) as reflected by a standard deviation of 4.91. The less lipophilic metabolites had ratios much closer to unity: THCC (mean 1.06, median 0.95, SD 0.61, range 0.28–4.05); OH-THC (mean 1.14, median 0.96, SD 0.55, range 0.51–3.18).

Linear regression analysis (r-squared) for peripheral vs. central THC, THCC, and OH-THC were 0.1943, 0.7443, and 0.9382, respectively. However, if the three highest concentrations of OH-THC (>20ng/mL) were deleted from the analysis, the r-squared value for this analyte fell to 0.4891. Additionally, there were five cases for which antemortem hospital blood samples were collected. The results for these cases are summarized in the table below:

Case	Date / Time	Blood	THC	THCC	OH-THC
1	11/17/2017 10:06	Antemortem	16	160	7.1
	11/18/2017 8:30	Central	18	100	5.4
	11/18/2017 8:30	Peripheral	>50	95	7
2	2/9/2018 14:30	Antemortem	0.94	7.5	ND
	2/10/2018 8:29	Peripheral	6.1	<5.0	ND
3	4/9/2018 21:35	Antemortem	26	94	7.3
	4/10/2018 10:50	Central	20	64	4.7
4	5/20/2018 unk	Antemortem	26	31	4.2
	5/21/2018 10:00	Central	12	40	6
	5/21/2018 10:00	Peripheral	11	23	3.3
5	7/3/2018 22:44	Antemortem	0.67	8.6	ND
	7/5/2018 9:45	Central	1.0	10	ND
	7/4/2018 18:10	Peripheral	3.9	6.2	ND

**Conclusions:** Postmortem peripheral blood THC concentrations exceeded central blood concentrations in 80% of cases studied. Additionally, in three out of four cases where antemortem blood was collected the day prior to peripheral blood, peripheral blood THC concentrations exceeded antemortem THC concentrations. Cannabinoid concentrations in postmortem blood can be subject to significant variability, and THC concentrations, in particular, should not be interpreted as if they were antemortem blood specimens.

### Cannabinoid, Postmortem, Variability

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### K71 A Comparison of Syringe Contents to Respective Biological Counterparts in 43 Medical Examiner Cases

*Sophia Brathwaite, BSc\*, Office of the Chief Medical Examiner, Washington, DC 20024; Lucas W. Zarwell, MFS, Washington, DC 20011; Samantha Tolliver, PhD, Office of the Chief Medical Examiner, Washington, DC; Chikarlo R. Leak, PhD, Office of the Chief Medical Examiner, Washington, DC 20024; Roger A. Mitchell, Jr., Office of the Chief Medical Examiner, Washington, DC 20024*

**Learning Overview:** At the end of this presentation, attendees will be better positioned to explore alternative strategies for determining the prevalence of New Psychoactive Substances (NPS).

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by encouraging toxicologists to evaluate scene paraphernalia in parallel with autopsy specimens from apparent accidental drug overdose cases. The correlation between the sets of data may provide toxicologically relevant information as well as prevalence information.

From March of 2017–present, the District of Columbia Medical Examiner has sought to preserve and solicit testing of syringe residues in an effort to identify emerging drugs of abuse. Results presented and discussed are from March 2017–March 2018.

Sixty-three syringes and other paraphernalia were collected from apparent accidental drug overdose investigative scenes in 43 medical examiner cases. In some instances, multiple syringes were collected per case. Two laboratories were employed. One laboratory analyzed the syringe residues for controlled substances and the second laboratory analyzed autopsy specimens for drugs of abuse.

First, a qualitative method developed to identify 21 fentanyl analogs, designer opioids, naloxone, and nalorphine by Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) was validated in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX). In this method, postmortem blood and/or urine were subjected to liquid extraction. A reverse phase gradient was applied using ammonium formate (5mM, pH 3) and 0.1% formic acid in acetonitrile to achieve chromatographic separation by use of an Waters® Acquity® UPLC BEH C18 2.1mm x 50mm, 1.7µm analytical column coupled to a Waters® Acquity® UPLC I-Class system. Mass spectral identification was conducted via a Waters® Acquity® TQ Detector.

The second laboratory implemented analysis of syringe contents for controlled substances via Cobalt Thiocyanate color testing followed by Gas Chromatography/Mass Spectrometry (GC/MS) testing. Both methods are consistent with the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) protocol. Bulk liquids and/or residues were emptied into appropriate containers followed by a syringe wash using a small volume of methanol (~250µL–500µL). If blood was present in the syringe barrel, chloroform was the solvent of choice. The organic layers of the extracts were then used to perform cobalt thiocyanate color testing and GC/MS analysis.

Of the 63 syringes (43 cases) that were collected between the periods, 76% of syringe contents correlate to postmortem blood and/or urine that contained fentanyl, a fentanyl analog, designer opioid, and/or opioid receptor antagonists. Of these syringes, the most common substance was fentanyl (64%), followed by furanyl fentanyl (28%). Other fentanyl analogs detected include para-fluoroisobutyryl/FIBF (8%), U-47700 (8%), methoxy acetyl fentanyl (4%), and despropionyl fentanyl (4%). Additionally, opiates and cutting agents, such as noscapine, caffeine, and diphenhydramine, were detected as well.

The analysis of paraphernalia can yield information of toxicological importance when trying to gain understanding about the prevalence of NPS when little to no information is present. However, the detection of the same compound in both paraphernalia and autopsy specimen is not guaranteed. These types of surveys may help the community better understand the relationship between metabolites and parent compounds. Although evaluated on a small scale, fentanyl and furanyl fentanyl seem to be prevalent in the drug overdose cases involved and, similarly, conclusions can thus be made about prevalence in Washington, DC. Knowledge about these substances can be used as a public health tool for education regarding misuse of these substances.

#### Fentanyl Analogs, Postmortem, Paraphernalia





### K72 Suicide by Pesticide? A Case Study of Fenobucarb Ingestion

*Marissa J. Finkelstein, MS\*, Miami-Dade Medical Examiner Department, Miami, FL 33136; Katherine L. Kenerson, MD, Miami-Dade Medical Examiner Department, Miami, FL 33136; George W. Hime, MS, Miami-Dade Medical Examiner Department, Miami, FL 33136; Diane Boland, PhD, Miami-Dade Medical Examiner Department, Miami, FL 33136*

**Learning Overview:** After attending this presentation, attendees will better understand: (1) the pathological and toxicological findings that indicate death due to the ingestion of 2-(1-methylpropyl) phenyl methylcarbamate (fenobucarb), (2) the symptoms of carbamate poisoning, and (3) how the autopsy results of carbamate pesticide ingestion differ from bleach/hydrofluoric acid ingestion.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the need for open lines of communication between pathologists and toxicologists to determine the cause of death in cases in which misleading information may be provided to investigators by family, hospital personnel, and first responders. This presentation will also impact the forensic science community by demonstrating the need for toxicologists to utilize antemortem clinical manifestations and autopsy findings to guide their route of analysis.

In December 2015, a 44-year-old Black male and his wife were involved in a heated, verbal and physical altercation at their residence. During the dispute, the intoxicated decedent discharged a handgun, causing his family to vacate the scene. He then went inside the residence and attempted suicide by ingesting an unknown caustic liquid. According to first responders, the liquid was hydrochloric acid, hydrofluoric acid, or bleach. Numerous attempts were made by the investigator to retrieve the unknown liquid from the terminal event scene. However, because the decedent was taken to the hospital, the bottle was never impounded by police or crime scene investigators.

The decedent was treated by fire rescue and taken to the hospital, where he expired three hours later. The decedent was conscious while being treated, but exhibited altered mental status, vomiting, and increased secretions. Upon arrival at the emergency room, the decedent presented with hypersalivation, respiratory failure, hypocalcemia, and increased potassium levels. The hospital staff consulted the Poison Control Center along with a hospital toxicologist. The toxicologist recommended aggressive calcium administration, a gastrointestinal endoscopy in less than 12 hours, and a calcium gel placement. Despite medical intervention, he went into cardiac arrest and expired in the emergency room.

Significant autopsy findings included the ingestion of 1.325L of a green-black, sludgy, granular fluid in the stomach, associated with staining of adjacent tissues, hemorrhage of retroperitoneal and mesenteric adipose tissues, hemorrhage of the soft tissues of the porta hepatis, left pleural effusion (140mL), and ascites (50mL). These findings were not characteristic of the ingestion of bleach or acid, which would have caused significant destruction of the stomach wall and soft tissues of the abdomen. Routine toxicological analysis detected ethanol (0.130%) in the antemortem blood, as well as diphenhydramine, lidocaine, ibuprofen, and naproxen in the gastric contents.

With no definitive cause of death, further toxicology testing was deemed necessary in this case. The pH of the gastric contents was tested to definitively rule out acid or bleach consumption, and the results indicated a physiologically normal pH of 2–3. Because of the autopsy findings and the decedent's symptoms prior to death, a screen to rule out organophosphate poisoning was performed. The gastric and small intestine contents were screened by Liquid-Liquid Extraction followed by Gas Chromatography/Mass Spectrometry (LLE-GC/MS) and 2-(1-methylpropyl) phenyl methylcarbamate (fenobucarb) was detected. Fenobucarb is a carbamate insecticide, and symptoms of carbamate poisoning include, but are not limited to, increased secretions, diarrhea, vomiting, confusion, respiratory depression, and cardiac arrest.

Based on the decedent's history, autopsy findings, and toxicology results, the forensic pathologist determined that the cause of death was ingestion of 2-(1-methylpropyl) phenyl methylcarbamate (fenobucarb), and the manner of death was suicide. This case demonstrates the necessity to consider all information, including clinical manifestations prior to death, autopsy findings, and toxicology results, in order to make an accurate determination as to cause and manner of death. This case also exemplifies that information provided to investigators from outside sources may not be accurate and may cause the investigation to head in the wrong direction.

#### Fenobucarb, Carbamate Pesticide Poisoning, Postmortem Toxicology



## K73 Death Due to Acute Nicotine Intoxication

Tiffany A. Hollenbeck, DO\*, Jackson County Medical Examiner's Office, Kansas City, MO 64108; Marius Tarau, MD, Kansas City, MO 64108; Lindsey J. Haldiman, DO, Jackson County Medical Examiner's Office, Kansas City, MO 64108

**Learning Overview:** After attending this presentation, attendees will be familiar with the toxic and lethal effects of nicotine, as well as the challenges in toxicological analysis of nicotine.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by highlighting the significant toxicity associated with liquid nicotine used to manufacture electronic cigarettes.

**Background:** Nicotine is a lipid soluble alkaloid substance that is extracted from the leaves of the *Nicotiana* plant.<sup>1</sup> Most commonly, nicotine is used in tobacco products such as cigarettes and chewing tobacco; it is found in some pesticides as well.<sup>2</sup> Nicotine is one of the most toxic and popular drugs of abuse; however, it rarely causes fatalities.<sup>1</sup> Electronic cigarettes were first introduced in 2004 and have gained significant popularity in recent years.<sup>3</sup> They are advertised to satisfy nicotine addiction without all the health hazards of inhaling tar and other carcinogens known to be present in traditional cigarettes. Liquid nicotine, the key ingredient in electronic cigarettes, poses a significant toxicological concern in that a very small ingested amount can be poisonous and life threatening.<sup>1-3</sup>

**Case:** A 23-year-old male was found by his roommate unresponsive, covered in vomit, in the bathroom of their residence. Emergency medical services were dispatched to the scene and resuscitative attempts were made for approximately 30 minutes before the subject was pronounced deceased. His roommate reported that he had seen the subject about 1 hour and 45 minutes prior and he did not appear to be intoxicated at the time. Of note, the subject had a bag which contained commercially available "stress relief-eucalyptus and spearmint" bath salts and three empty and apparently unused syringes that were, therefore, not collected for analysis. A nearly full package of cigarettes was found near the decedent, but no other nicotine products were identified at the scene. Autopsy revealed marked pulmonary edema and congestion of the kidneys and liver. There was no evidence of trauma identified. A drug screen performed on heart blood was positive for nicotine and amphetamines. Toxicological analysis of femoral blood revealed nicotine at a concentration of 2,400ng/ml and cotinine (a metabolite of nicotine) at a level of 210ng/ml, (limit of detection for nicotine and cotinine is 5.0ng/mL and 10.0ng/mL, respectively); these concentrations support the acute nature of the intoxication. Ethanol was also detected at a level of 13mg/dL. Amphetamines were not confirmed by liquid chromatography/mass spectrometry with a limit of detection at 10ng/mL. A novel psychoactive substances screen for bath salts was performed by National Medical Services (NMS) lab and was negative.

**Conclusion:** Liquid nicotine, used to fill electronic cigarettes, contains very high concentrations of nicotine, up to 36.6mg/mL in a refill solution, which can be toxic or lethal when consumed in even a small dose.<sup>4</sup> It is of great toxicological concern due to the ease of access to the substance with no strict control over distribution.<sup>1</sup> The estimated lethal dose of nicotine is about 30mg–60mg in an adult.<sup>5</sup> Effects of nicotine on the body are complex, with small doses causing a stimulatory effect (nausea, vomiting, dizziness, miosis, tachycardia, hypertension, sweating, and salivation) and lethal doses causing a depressive effect (prostration, convulsions, respiratory paralysis, and cardiac arrhythmias).<sup>5</sup> Other nicotine-related fatalities have been reported, primarily due to accidental exposure related to harvesting nicotine from the plant.<sup>2</sup> Few case reports have described ingestion of liquid nicotine to commit suicide, although the majority of these cases have other substances in addition to nicotine detected postmortem; most commonly a significant amount of alcohol and/or opioids, which contributes to the depressive effect on the central nervous system.<sup>1</sup> Although liquid nicotine was not located on scene, based on a literature review, the most likely method of sustaining such a high nicotine level in such a short period of time is either by ingestion or injection of a concentrated form of nicotine, as found in e-cigarette refills. This case is interesting in that the scene investigation suggests that the subject may have injected the nicotine (which is extremely rare), as opposed to ingesting it.

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### Nicotine Intoxication, Electronic Cigarette, Liquid Nicotine



### K74 Validation of the Neogen® Enzyme-Linked Immuno-Sorbent Assay (ELISA) Benzodiazepine Kit Using Clonazepam as the Target Molecule for Blood and Urine

Grayce Behnke, BS\*, Export, PA 15632; Nicholas B. Tiscione, MS, West Palm Beach, FL 33406; John Rakus, PhD, Marshall University, Huntington, WV 25755; Lauren L. Richards-Waugh, PhD, Marshall University, Huntington, WV 25701

**Learning Overview:** After attending this presentation, attendees will understand: (1) the effectiveness of the Neogen® ELISA Benzodiazepine kit for screening whole blood and urine specimens using clonazepam as the target molecule, and (2) the challenges with following the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology Laboratories.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating the validation of an ELISA method for the screening of a broad range of benzodiazepines.

**Objective:** The validation of a semi-quantitative method for the rapid screening of whole blood and urine specimens by a Dynex DSX® Automated ELISA System using the Neogen® Benzodiazepine kit and clonazepam, rather than oxazepam, as the target molecule.

**Method:** Neogen® Benzodiazepine kit assay instructions for incubation times and reagent volumes were followed. The amount of sample added was increased from the recommended 10µL to 20µL. Whole blood samples were diluted 1:5 with Neogen® EIA buffer offline. Urine samples were diluted 1:10 with Neogen® EIA buffer online by the instrument. Performance of the assay was evaluated at one decision point for each matrix: 10ng/mL in whole blood and 25ng/mL in urine. An in-house validation protocol based on the SWGTOX standard practices was followed for the validation of the assay, which included the evaluation of sensitivity, precision, specificity, carryover, drift, ruggedness/robustness, and a case sample comparison.

**Results:** The theoretical Limit Of Detection (LOD) for clonazepam was calculated to be 2ng/mL in blood and 8ng/mL in urine. The experimental LOD for clonazepam was determined to be at least 5ng/mL in blood and 10ng/mL in urine. Precision was evaluated using the mean of three replicates from five separate runs ( $n=15$ ) at the decision point and at concentrations  $\pm 50\%$  and  $\pm 100\%$  of the decision point. Although the method was optimized, and precision was demonstrated at each level (coefficient of variation  $< 7.6\%$ ), the current SWGTOX validation requirements for a valid decision point were not fulfilled. Furthermore, the urine matrix did not meet the proposed revision of the SWGTOX requirements for determining a valid decision point promulgated by the Toxicology Subcommittee of the Organization of Scientific Area Committees for Forensic Science. Cross reactivity was observed with all 29 low- and high-dose benzodiazepines analyzed. If the cross reactivity of a compound was less than 100%, then the precision for the detection of that compound was evaluated. No carryover or drift was observed. The method proved to be both rugged and robust. Case sample comparison results were comparable to those obtained when the samples were initially screened using oxazepam as the target molecule. One false negative by both the original ELISA with oxazepam and reanalysis with clonazepam was identified by confirmation with liquid chromatography/tandem mass spectrometry.

**Conclusion:** The Neogen® Benzodiazepine kit using clonazepam as the target molecule exhibited high cross reactivity for 29 different low- and high-dose benzodiazepines and demonstrated excellent precision and sensitivity in both whole blood and urine, making it an efficient and reliable method to screen blood and urine specimens for benzodiazepines, even though it did not fulfill current SWGTOX validation requirements for a valid decision point.

**Validation, ELISA, Benzodiazepine**



### **K75 Quantitative Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Analysis of 39 Fentanyl Analogs and Metabolites in Blood, Urine, and Oral Fluid**

*Marilyn A. Huestis, PhD\*, Huestis & Smith Toxicology, LLC, Severna Park, MD 21146; Dominic Andrada, MS, Thermo Fisher Scientific, Sunnyvale, CA 94085; Rory M. Doyle, PhD, Thermo Fisher, Somerset, NJ 08873*

**Learning Overview:** After attending this presentation, attendees will know that many fentanyl analogs and metabolites can be identified and quantified in blood, oral fluid, and urine in a single sensitive and specific LC/MS/MS procedure.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how to identify and quantify fentanyl analogs in diverse biological matrices to document cause of death, the drugs involved with driving under the influence of drugs, and substance abuse.

Fentanyl and its novel psychoactive substance analogs are of strong interest due to increased potency, increased abuse liability, and the large number of intoxications and deaths occurring in the United States. Fentanyls are opioids with a rapid onset of effects that are injected or absorbed from a skin patch or oromucosally from an oral lollipop or buccal tablet. Fentanyls activate the  $\mu$ -opioid receptor and may be as much as 10,000 times more potent than morphine. Food and Drug Administration-approved synthetic fentanyls include pain medications and anesthesia agents. Recently, dozens of fentanyl analogs were introduced by clandestine laboratories located in China and other countries, resulting in many overdoses and deaths. Clinical hospital laboratories and local, state, and federal forensic toxicology laboratories need to identify the novel psychoactive substances responsible for these adverse events to alert public health authorities, first responders, and drug users about the presence of a new toxic drug, as well as to provide psychiatric follow up for the drug user. This fentanyl analytical method was developed and optimized for accurate and robust drug analysis, while also demonstrating the challenges associated with investigating multiple compounds of similar structure and physicochemical properties.

A Thermo Scientific™ Vanquish™ HPLC system and a Thermo Scientific™ TSQ Quantis™ tandem mass spectrometer in positive electrospray mode were utilized for the analysis. Sample size was only 100 $\mu$ L urine, oral fluid, or blood. The selected column was a Thermo Scientific™ Accucore™ C18 100mm x 2.1mm, 1.5 $\mu$ m column heated to 50°C, with 5 $\mu$ L injected. Mobile phase A was a water: methanol mixture containing 0.01% formic acid and 2mM ammonium formate and mobile phase B was methanol. The gradient began at 90% A and 10% B ending at 8.5min with 2% A and 98% B. Analyte elution occurred from 0.52min (NPP) to 7.2min (Valeryl Fentanyl). Baseline chromatographic separation was achieved for all analytes within a total run time of 10min.

Mass spectrometer settings were: vaporizer temperature: 475°C, ion transfer tube temperature 300°C, sheath gas: 70, aux gas: 10, sweep gas: 0, spray voltage: positive ion (V): 500, Q1/Q2 resolution: 0.7/0.7 (FWHM), cycle time (sec): 0.6, CID gas (mTorr) 2 and chromatographic peak width 6secs. Collision energies were optimized for each transition and ranged from 10V to 45V. Quantitative analysis was performed with Selective Reaction Monitoring (SRM) transition pairs for each analyte and internal standard. Reference materials for method validation were from UTAK.

Sample preparation techniques included protein precipitation for blood samples and simple dilution of urine and oral fluid samples, enabling high throughput analysis. Limits Of Quantification (LOQ) in blood ranged from 5pg/mL (acetyl norfentanyl) to 100pg/mL (remifentanyl), in oral fluid 10pg/mL (norfentanyl) to 100pg/mL ( $\beta$ -hydroxythiofentanyl), and in urine 25pg/mL (4-Anilino-4-piperidine) to 250pg/mL (N-methyl norcarfentanyl). Good linearity and reproducibility were obtained for all fentanyl analogs and metabolites with a coefficient of determination  $R^2 > 0.98$  or better for all drugs in the different matrices. Excellent imprecision and accuracy were achieved (CV < 15%) for all compounds in all matrices. The method achieved sensitive results for the analysis of 39 new fentanyl analogs with subtle structural differences and demonstrated the versatility of the mass spectrometer to consistently quantify multiple, closely related drugs at pg/mL concentrations.

#### **Fentanyl Analogs, LC/MS/MS, Blood, Oral Fluid, and Urine**



### K76 The Identification of Drug Metabolites in Adulterated Urine Samples Using Direct Analysis in Real Time-Time Of Flight/Mass Spectrometry (DART®-TOF/MS)

Bianca E. Olivieri\*, Lakeland, FL 33810; Mark Maric, PhD, Orlando, FL 32816; Candice Bridge, PhD, National Center for Forensic Science, University of Central Florida, Orlando, FL 32816

**Learning Overview:** The goal of this presentation is to discuss complications that arise with adulteration of urine samples in drug analysis and how to analyze the samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new analytical technique to screen for drugs and their metabolites as well as detecting the presence of adulterants in urinalysis.

With the increased technology to identify drugs and their metabolites in urine screenings, more individuals are using forms of adulterants to evade positive results.<sup>1</sup> In a previous study, it was concluded that certain adulterants (e.g., bleach, Drano®, and eye drops) were able to create false-negatives when using a common screening procedure comprised of Enzyme-Linked Immunosorbent Assay (ELISA) and adulterant test strips (Intect® 7 and AdultaCheck® 6).<sup>2</sup> To circumvent false negatives using immunoassay techniques and to avoid subjectivity through color detection, this study proposes using DART®-TOF/MS to identify not only the drug of interest, but also any adulterants present within the sample. DART®-TOF/MS is an ambient ionization technique that has been shown through previous literature to have a high-resolution mass spectra and rapid analysis with minimal sample preparation.<sup>3</sup> DART®-TOF/MS has been used previously with bodily fluids and has shown to identify endogenous substances (e.g., creatinine) as well as exogenous substances (e.g., prescription drugs).<sup>3</sup>

For this study urine samples were collected anonymously by volunteers under Institutional Review Board (IRB) NO: SBE-16-12568. Volunteers also provided information on the study survey regarding drug use within the past week, which was then used to identify samples containing the drugs of interest and their metabolites. Samples collected contained tetrahydrocannabinol (11-nor-carboxy-delta-9-THC), cocaine (benzoyl ecgonine), amphetamines (d-amphetamine), and benzodiazepines (lorazepam, lorazepam glucuronide). These samples were then adulterated with bleach, vinegar, eye drops, Drano®, nitrite, table salt, and hand sanitizer at different concentrations (i.e., 5, 10, 25, 50% v/v or w/v). Adulterated samples were prepared for analysis using IonSense® SPE-it™ fiber kits, which uses a solid-phase micro extraction technique to isolate the analytes from components in the urine matrix. After sample preparation, Solid-Phase Microextraction (SPME) fiber strips were analyzed in the positive ionization mode on DART®-TOF/MS by placing the fiber into the ionization stream.

Initial data supports the use of DART®-TOF/MS as an analytical screening technique to identify drug metabolites and the presence of adulterants. The peaks observed during DART®-TOF/MS analysis indicated adulteration at low concentrations (i.e., 5% v/v) does not mask the presence of the drug/metabolite, unlike the combined immunoassay and adulterant test strip screening techniques, which were unable to detect adulteration at these low concentrations, as well the target drug/metabolite in some instances. Bleach was one adulterant that was easily observed in analysis, due to the presence of chlorinated peaks. A urine sample containing amphetamines and its metabolites was adulterated with bleach at the same five concentrations. As the concentration of bleach increased from the unadulterated amphetamine sample to a 50% v/v adulterant concentration, it was more difficult to observe the signal from the protonated amphetamine. However, the adulterant was still observed. Amphetamine was readily observed up to 10% v/v adulteration. Additional peaks, such as metabolites and fragments of the parent, were also observed in the resulting spectra. This trend was observed for the drugs in other urine samples that were exposed by different adulterants. Tampering of urine samples by adulteration requires the sample to be flagged and can lead to consequences for the individual; however, this cannot be achieved by current screening methods at low adulterant concentrations.

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#### DART®-TOF/MS, SPME, Urine Adulterants

### K77 The Quantification of 4-Fluoroamphetamine, 4-Fluoromethamphetamine, 4-Chloroamphetamine, and 4-Chloromethamphetamine in Postmortem Blood and Urine by Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS)

*Chu-An Yang, MS\*, New Taipei City 235, TAIWAN, REPUBLIC OF CHINA; Hsiu-Chuan Liu, MS, Taipei, TAIWAN, REPUBLIC OF CHINA; Ray H. Liu, PhD, Forensic Science Review, Vancouver, WA 98685; Dong-Liang Lin, PhD, Institute of Forensic Medicine, New Taipei City 23548, TAIWAN, REPUBLIC OF CHINA*

**Learning Overview:** The goal of this presentation is to provide insights of a highly sensitive UHPLC-MS/MS approach for the analysis of four New Psychoactive Substances (NPS)—4-fluoroamphetamine, 4-fluoromethamphetamine, 4-chloroamphetamine, and 4-chloromethamphetamine—in blood and urine specimens following a liquid-liquid extraction sample preparation protocol.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through the development and validation of the UHPLC-MS/MS method to improve the identification and quantification of certain NPS in postmortem specimens.

4-fluoroamphetamine, 4-fluoromethamphetamine, 4-chloroamphetamine, and 4-chloromethamphetamine are substituted phenethylamine-type synthetic NPS, with amphetamine- and methamphetamine-like structural feature. Reportedly, 4-fluoroamphetamine and 4-fluoromethamphetamine produce mainly sympathomimetic effects and exhibit entactogenic properties, while 4-chloroamphetamine and 4-chloromethamphetamine stimulate the central nervous system, with a longer half-life than their fluoro-counterparts. These phenethylamine-type NPS have gained popularity on the illegal market and have been detected in specimens from many death cases in Taiwan during the past six years.

Blood or urine (0.5mL) were mixed with sodium hydroxide and extracted with ethyl acetate. The extracts were evaporated and reconstituted in the mobile phase (initial gradient composition) for injection onto the UHPLC-MS/MS system. Deuterated analogs of the analytes were used as internal standards. Chromatographic separation was achieved using an Agilent® ZORBAX® SB-Aq (100mm × 2.1mm i.d., 1.8-µm particle) analytical column at 50°C. The mobile phase included 0.1% formic acid (v/v) in water (A) and methanol (B), with a flow rate of 0.32mL/min. The initial gradient composition (A/B 90:10, v/v) was decreased to 0% A in 6min; then increased to 90% A in 1min. Parameters for mass spectrometric analysis included: (1) Agilent® Jet Stream Technology electrospray ionization in positive-ion Multiple Reaction Monitoring (MRM) mode, (2) optimized collision energy levels for selected precursor ions, and (3) monitoring two transitions for analytes and internal standards.

Validity of the methodology was assessed using drug-free blood and urine that were fortified with 100ng/mL–2000ng/mL of the analytes. The following analytical parameters were obtained: (1) average extraction recovery, derived from five different sources of blood and urine, was higher than 75%; (2) matrix effect (ion suppression) was observed, except for blood samples of 4-chloroamphetamine at the 100ng/mL and 250ng/mL concentration levels, but was adequately compensated for by using respective deuterated internal standards; (3) intra-/inter-day precision (%CV) and accuracy ranges for blood were 0.6%–7.0%/3.7%–10.2% and 85%–109%/93%–105%, while the corresponding ranges for urine were 0.8%–5.1%/3.5%–17.7% and 86%–110%/92%–106%; (4) calibration linearity ( $r^2$ ) for all analytes were >0.997; and (5) the limits of detection for 4-fluoroamphetamine, 4-fluoromethamphetamine, 4-chloroamphetamine, and 4-chloromethamphetamine in urine and blood were 2.5ng/mL, 0.5ng/mL, 1ng/mL, 5ng/mL and 0.5ng/mL, 0.5ng/mL, 2.5ng/mL, 5ng/mL, respectively.

In conclusion, this relatively simple protocol was found effective and reliable for routine identification and quantification of these NPS in blood and urine. This method was applied to the analysis of a limited number of postmortem specimens (one blood and four urine) from forensic cases during the April-July 2018 period. 4-chloroamphetamine and 4-chloromethamphetamine were found in the blood and the urine specimens from the same case, with 233ng/mL and 11,151ng/mL for blood and 807ng/mL and 185,095ng/mL for urine. 4-fluoroamphetamine was found in the other three urine specimens with the concentrations ranging from 183ng/mL to 2,526ng/mL.

#### Phenylethylamine Derivatives, Postmortem, UHPLC-MS/MS

## K78 Staying Relevant in an Ever-Changing Climate: The Development and Validation of a Confirmatory Qualitative Synthetic Cannabinoid Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Method in Human Whole-Blood

Rebecca A. Mastrovito, MS\*, NMS Labs, Willow Grove, PA 19090; Stephanie Kumor, MA, NMS Labs, Willow Grove, PA 19090; Joseph Homan, MS, NMS Labs, Willow Grove, PA 19090; Parul Shah, BS, NMS Labs, Willow Grove, PA 19090; Denise Nicole Schiller, MSFS, Bristol, PA 19007; Sherri L. Kacinko, PhD, Willow Grove, PA 19090; Barry K. Logan, PhD, NMS Labs/Center for Forensic Science Research and Education, Willow Grove, PA 19090

**Learning Overview:** After attending this presentation, attendees will be able to discuss the designer-drug class of synthetic cannabinoids with a focus on the latest trends and will be able to undertake the development and validation of a confirmatory analytical assay using LC/MS/MS for their detection in biological fluids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the abuse and toxicity of synthetic cannabinoids as well as their detection in biological samples.

Since 2009, synthetic cannabinoids have presented a challenge to toxicology laboratories. As new compounds become available within the recreational drug market, labs need to update their analytical methods to stay relevant. The development and validation of quantitative methods can be a long and demanding process, especially when there is a lack of deuterated internal standards for every analyte in the panel. Developing a qualitative confirmation method allows faster updates to incorporate new compounds as they emerge, due to the less-stringent criteria. Synthetic cannabinoids used in abused products are continually changing with slight structural alterations to circumvent drug control regulations created for earlier generation analogs. Since these positional isomers have identical molecular weights and very similar fragmentation patterns, they are indistinguishable by MS/MS detectors.

The proposed scope contained 27 analytes, including 10 new analytes. Concentrations range from 0.05ng/mL–1.0ng/mL.

CURRENT		NEW
CUMYL-THPINACA	FUB-JWH-018	FDU-PB-22
MDMB-FUBINACA+	MMB-CHMINACA	5-fluoro-NPB-22
FUB-AKB48	5-fluoro-AMB	5-fluoro-MDMB-PICA
MA-CHMINACA	FUB-JWH-018	5-fluoro AEB*
5-fluoro-ADB*	MMB-CHMINACA	4-cyano CUMYL BUTINACA
NM-2201	AB-FUBINACA	EMB-FUBINACA+
FUB-AMB	ADB-FUBINACA	ADB-FUBICA
MO-CHMINACA	AB-CHMINACA	MDMB-FUBICA
MDMB-CHMCZCA	ADB-CHMINACA	MMB-FUBICA
MMB-CHMICA		5-fluoro-EDMB-PINACA

\* 5-fluoro-ADB and 5-fluoro AEB are reported as pairs  
+ MDMB FUBINACA and EMB FUBINACA are reported as pairs

This assay was developed to detect and qualitatively identify synthetic cannabinoid compounds in whole blood. Whole blood was fortified with internal standard, pH adjustment with 0.1 M TRIS buffer pH 10.2, and single-step liquid/liquid extractions. Separation was achieved using two different chromatographic methods run on an Acquity® UPLC BEH C18 (100mm x 2.1mm, 1.7-micron) column coupled with a VanGuard® BEH C18 1.7-micron guard column with mobile phases consisting of 0.1% formic acid in deionized water and an acetonitrile/methanol mixture. Analytes were detected using positive-ion electrospray MS/MS on a Waters® TQS MS/MS coupled with a Waters® Acquity® Ultra Performance LC System.

The methods were fully validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. During method development, it was demonstrated that running a calibration curve improved the precision around the cut-off concentration. However, the quantitative controls for several analytes did not meet the stringent requirements required for the quantitative validation, usually because of the lack of a labeled internal standard. Therefore, the method was validated qualitatively according to laboratory Standard Operating Procedure (SOP), including the evaluation of the cut-off concentration, sensitivity/specificity, carryover, matrix effect, interfering substances, and stability. Linearity was established using five calibrators. Replicates ( $n=5$ ) at each concentration were analyzed and the correlation coefficient was  $>0.99$  for all analytes. All ten synthetic cannabinoids were measured at three different concentrations to give precision  $\leq 15\%$  CV and accuracy  $\pm 15\%$  for both within- and between-run experiments. Stability experiments ( $n=6$ ) indicated that the synthetic cannabinoids listed above were stable in blood for up to two days at room temperature, and for at least 30 days if kept refrigerated or frozen. Exceptions were MMB FUBICA, which was not stable at room temperature, and 5-fluoro EDMB-PINACA was only stable for seven days refrigerated.

Four hundred fifty-six blood samples that had screened positive for synthetic cannabinoids using an LC/MS/MS screening method were tested using this updated method. Twenty cases tested positive for one or more of the newly added cannabinoids. Seven cases tested positive for 4-cyano CUMYL BUTINACA and three samples were confirmed positive for MMB-FUBICA in combination with other new designer cannabinoids (5-fluoro MDMB-PICA and 4-cyano CUMYL BUTINACA). Of the cases with demographic information available, the median age of the subjects was 33, and included 15 males and 1 female.

### Synthetic Cannabinoids, Novel Psychoactive Substances, Validation

### K79 The Development and Validation of an Analytical Method for the Identification of New Psychoactive Substances (NPS) Using the Retention Index and Gas Chromatography/Mass Spectrometry (GC/MS)

Gabriella H. Giudice, BSc\*, Brazilian Health Regulatory Agency, Brasília 71205-050, BRAZIL; Angelo H. Lira Machado, DSc, University of Brasília, Brasília, Distrito Federal 70910-900, BRAZIL; Jorge J. Zacca, Setor Sudoeste, Brasília, DF 70673-046, BRAZIL

**Learning Overview:** After attending this presentation, attendees will understand NPS and the application of some principles of GC and retention indices to identify controlled substances.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by proposing a validated GC/MS analytical method that could eliminate or minimize the need for NPS analytical standards to perform systematic illicit drug identification. This method is suitable for laboratorial forensic routine after a simple validation protocol.

The United Nations Office on Drugs and Crime (UNODC) defines NPS as “substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat.”<sup>1</sup> The rate at which these substances are introduced into the market poses a challenge to both regulatory and law enforcement institutions.

In 2016, Brazil adopted a generic classification for the synthetic cannabinoid group, the most widespread NPS class worldwide, to anticipate controls upon the arrival of new substances in the country. After that, a generic classification for synthetic cathinones was also included as a controlled group.

Additionally, forensic science institutes in Brazil have faced analytical challenges when dealing with the unequivocal identification of these new seized substances. Some of these challenges are related to the unavailability of analytical chemical standards due to changes to the functional groups in the molecule during synthesis, the slow rate of commercial production in comparison with drug market changes, high costs, etc.

To work around this problem, the present work benefits from Kováts Retention Indices, which make use of a homologous n-alkane series to both reference and normalize retention times. This type of retention index has been used for high accuracy chemical compound identification with relative standard deviations smaller than 0.35% in the case of illicit drugs.<sup>2,3</sup> It allows for inter-laboratory applicability and verification, and it is robust under different gas flow conditions.<sup>4</sup>

The proposed GC/MS method has been validated with resolution, robustness, and precision measures as figures of merit. A mixture of 21 drugs have been tested to cover as much of the elution time window as possible. The mix was composed of three synthetic cannabinoids (AM-2201, MAM-2201, JWH-081), one cathinone (N-ethylpentylone), three phenylethylamines (5-MAPB, 2-FA, 25C-NBOMe), two piperazines (o-CPP, p-CPP), one opioid (U-47700), one tryptamine (5-MeO-MiPT), one aminoindane (5-IAI), one of vegetal origin (*Salvia divinorum*), methiopropamine, and seven traditional drugs (THC, heroin, amphetamine, methamphetamine, MDMA, cocaine, ephedrine). The GC/MS method has shown to present enough resolution to separate the majority of compounds. One coelution occurred between methiopropamine and methamphetamine. In such cases, mass spectra interpretation may be used in compound disambiguation. The lowest resolution (1.09) was obtained for N-ethylpentylone. The smallest Kováts index difference (4.9) occurred between amphetamine and 2-FA.

These preliminary results seem to indicate the association of Kováts indices with GC/MS as an efficient, low-cost, easy-to-adapt alternative to forensic labs in both the identification and categorization of NPS.

#### Reference(s):

1. <https://www.unodc.org/LSS/Page/NPS>.
2. Imhoi Koo, Xue Shi, Seongho Kim, Xiang Zhanga. Compound Identification Using Retention Index for Analysis of Gas Chromatography–Mass Spectrometry Data. *Journal of Chromatography A*. 2014; 1337: 202-210.
3. Kelly K, Bell S. Evaluation of the Reproducibility and Repeatability of GCMS Retention Indices and Mass Spectra of Novel Psychoactive Substances. *Forensic Chemistry*. 2018; 7: 10–18.
4. V.I. Babushok, P.J. Linstrom, J.J. Reed, I.G. Zenkevich, R.L. Brown, W.G. Mallard, S.E. Stein. Development of a Database of Gas Chromatographic Retention Properties of Organic Compounds. *Journal of Chromatography A*. 2007; 1157:414-421.

**New Psychoactive Substances, Gas Chromatography/Mass Spectrometry, Kováts Retention Indices**





### **K80 Postmortem Pediatric Forensic Toxicology**

*Robert A. Middleberg, PhD, NMS Labs, Willow Grove, PA 19090; Nikolas P. Lemos, PhD, University of California-San Francisco, Palm Springs, CA 92262-6451; Sarah E. Avedschmidt, MD\*, University of Michigan, Ann Arbor, MI 48109; Alexander Robert W. Forrest, LLM\*, School of Law, Sheffield, South Yorkshire S3 7ND, UNITED KINGDOM; David R. Fowler, MD\*, Office of the Chief Medical Examiner, Baltimore, MD 21223; Greg Vincent, MD\*, Farmington, CT 06032*

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**Learning Overview:** After attending this presentation, attendees will understand the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both a general and case-specific sense.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

During this 19<sup>th</sup> Annual Special Session within the Toxicology Section, pediatric cases involving toxicological findings will be discussed. As a relative dearth of interpretive information involving toxicological findings exists in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation or issue-specific concern, including pharmacokinetic data and other relevant ancillary information, followed by audience participation to provide interpretive clarity around case-specific impacts of the toxicological findings. This session, attended by various sections of the Academy, allows for various perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause of death in children.

Four cases will be presented that highlight the difficulty in assessing the role of toxicants in each case or the lengths one must go to in some cases. Sarah Avedschmidt, MD, David Fowler, MD, Robert Forrest, LLM, and Greg Vincent, MD, will be reviewing cases from their experiences as forensic pathologists that highlight the issues and confounders in the pediatric population.

Dr. Avedschmidt will be discussing a case of a 4-year-old with Cri du Chat and sickle cell disease who expired in an emergency room. The postmortem toxicology demonstrated the presence of oxycodone. The steps taken to determine the role of the oxycodone in the death will be examined in the face of known pathologies, a number of other factors, and the ever-present unknowns.

Dr. Fowler will draw on his years of experience as a forensic pathologist in an inner city to discuss cases of note.

Dr. Forrest will dive deep into a 19<sup>th</sup>-century case involving strychnine, the initial diagnosis, and how a conclusion was reached given the availability of scientific data at the time. He will also discuss the likelihood of a proper diagnosis if a child were to present with similar signs and symptoms today.

Dr. Vincent will bring forward a case involving the death of an 8-year-old in a fire and how failure to perform proper toxicological testing may lead to an overlooked cause of death. The twists and turns of the case are both fascinating and caution-bearing.

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### **Pediatric, Toxicology, Postmortem**



### K81 Next-Generation Drug Surveillance in Public Health and Medicolegal Death Investigations

*Jeri D. Roper-Miller, PhD\**, RTI International, Research Triangle Park, NC 27709; *Kemp Chester, MA\**, White House Office of National Drug Control Policy, Washington, DC 20503; *M.J. Menendez, JD\**, Organized Crime Drug Enforcement Task Force, Washington, DC 20530; *Jeffrey R. Locke, JD\**, Springfield, VA 22150; *DeMia P. Pressley, MS\**, Drug Enforcement Administration, Springfield, VA 22152; *Conner Brooks, MSc\**, Bureau of Justice Statistics, Washington, DC 20001; *Margaret Warner, PhD\**, Center for Disease Control and Prevention, National Center Health Statistics, Hyattsville, MD 20912; *Andrew M. Baker, MD\**, Hennepin County Medical Examiner Office, Minneapolis, MN 55415; *Barry K. Logan, PhD\**, NMS Labs/Center for Forensic Science Research and Education, Willow Grove, PA 19090

**Learning Overview:** After attending this presentation, attendees will have an understanding of federal- and state-level efforts to enhance and implement drug surveillance programs to strengthen investigations involving drug-related deaths in the United States and promulgate information about the needs of the Medicolegal Death Investigations (MDI) community to facilitate their work. Attendees will also have gained evidence-based knowledge regarding infrastructure-related challenges faced by medical examiners, coroners, and their forensic laboratory services; acquired perspectives about lessons learned from local, state, and federal efforts working with and within the MDI community; and learned about resources that are in place to overcome some of these obstacles, including tangible resources, changing laws, and advances in technology, science, and availability of key data.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating how these national and state-wide data can improve our understanding of the state of medicolegal death investigation systems, highlighting valuable tools for public safety and public health, and sharing of these analytical data can facilitate comprehensive public health and death investigation efforts.

Investigations of drug-related deaths in the United States is at an unsurpassed critical “breaking point” that calls for enhanced, integrated sentinel drug surveillance efforts. Many surveillance efforts at a federal and state level are progressively relying on multidisciplinary teams to evaluate emerging drug trends; historical, current, and potential patterns of abuse; safety or dependence liability; public health risks and lethality; and analytical characteristics.<sup>1</sup>

In 2015, nearly 2.5 million people died in the United States.<sup>2</sup> Of these, approximately one million were referred to the nation’s Medical Examiner and Coroner offices (MECs), which accepted about half of these cases.<sup>3</sup> MEC caseloads are at an all-time high because many of these deaths require a complex, in-depth investigation.<sup>4</sup> Drug overdose deaths require significant resources to investigate, and the exponential escalation of drug-related deaths in the United States have led to national efforts to implement more effective and timely awareness of the epidemiology, drug statistics, and unprecedented challenges that burden MECs. In general, there is limited information about the state of the MDI community on a national scale concerning drug surveillance.

Several national MDI stakeholder meetings have focused on multiple federal and state agency efforts within the MDI community. It is important to highlight what these agencies are doing, how their work ties together and how they are collaborating, and how MDI practitioners are finding value in and implementing better practices using data from these efforts.

In summary, this special session will discuss the needs, progress, and current programs that will assist the forensic science community in achieving the next generational advancement of drug surveillance in MDIs.

#### Reference(s):

1. DEA, Controlled Substances Act, Section 201 (c), [21 U.S.C. § 811 (c), 2015.
2. CDC WONDER, 2017.
3. National Science and Technology Council, 2016.
4. CDC NVSS, 2016.

#### Drug Surveillance, Drug Overdose, Medicolegal Death Investigations



### LW1 Doyle: The Bitemark Case That Started It All!

*Roger D. Metcalf, DDS, JD\*, Tarrant County, Fort Worth, TX 76179; Janice W. Klim-Lemann, DDS, Redlands, CA 92373*

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**Learning Overview:** The goal of this presentation is to provide some background history about the seminal bitemark case in United States forensic odontology and to discuss its subsequent ramifications.

**Impact on the Forensic Science Community:** After attending this presentation, attendees will have a better appreciation of the *Doyle* bitemark case.<sup>1</sup> This presentation will impact the forensic science community by providing some historical background to the field of bitemark analysis.

James A. “Jimmy” Doyle was arrested in west Texas, not far from Abilene, in December of 1952 for public intoxication. The night before Doyle’s arrest someone had broken into Oscar Peacock’s grocery store in the small town of Aspermonte, and had stolen a number of items, including two bottles of whiskey and 13 silver dollars. In addition, a “large block of cheese” was found left on a countertop in the store, and it was noted there were “pronounced teeth marks” in the cheese.

During routine inventory of his personal property at the jail, Doyle had been found to have had 13 silver dollars in his pocket. So, while Doyle was incarcerated, on a “hunch,” Sheriff Frazier requested that he bite into another similar block of cheese in order to make an exemplar for comparison purposes. Doyle readily agreed and did so.

The cheeses were sent for analysis by a novel-at-the-time procedure and were examined by a Texas Department of Public Safety firearms and tool mark examiner, Mr. Taylor. The cheesy marks were also evaluated by a long-time, well-respected member of the Texas State Board of Dental Examiners, William Kemp, DDS, of Haskell, TX.

Mr. Doyle was charged with and convicted of burglary in a jury trial in Stonewall County. Mr. Doyle then applied for *certiorari* with the Texas Court of Criminal Appeals, Texas’ court-of-last-resort for criminal cases, and the Court agreed to hear his case. Doyle claimed on appeal that the request from Sheriff Frazier to make an exemplar bite in the second block of cheese violated his right against self-incrimination, because he had not received the pertinent warning required by statute in Texas at that time (predating *Miranda* by more than a decade). The Court did not find Doyle’s reasoning persuasive, and the verdict was upheld.

*Doyle* is Texas’ first known bitemark case and the first known *reported* (in the legal sense) bitemark case in the United States. The case is often cited by odontologists, but many may not know the interesting “back story.” This presentation will provide greater understanding about the history of a very important case and an appreciation of subsequent developments.

Please note that this case report was first presented in the Odontology Section Scientific Session of the 2016 AAFS Annual Scientific Meeting.

**Reference(s):**

<sup>1</sup>. *Doyle v. State of Texas*, 263 S.W.2d 779, Tex.Crim.App. 1954.

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**Bitemark, History, Cheese**



### LW2 Cosmic Forensics: Can the Sky Fall on Our Heads?

*Annarita Franza, PhD\*, Department of Experimental & Clinical, Florence, ITALY; Vincenzo Lusa, JD\*, Rome 00151, ITALY; Mario Di Martino, PhD, Italian National Institute for Astrophysics, Pino Torinese 10025, ITALY; Vanni Moggi Cecchi, PhD, Natural History Museum, University of Florence, Florence 50122, ITALY*

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**Learning Overview:** The goal of this presentation is to familiarize attendees with the role of forensic sciences in geosciences and space research.

**Impact on the Forensic Science Community:** This presentation will impact the forensic scientific community by providing useful information to those engaged in the study of extraterrestrial materials (i.e., meteorites, micrometeorites, and interplanetary dust particles) in a judicial contest. This presentation will focus primarily on meteorites, which have been documented to strike structures and objects or injure human beings.

Despite having been the subject of mineralogical, chemical, astronomical, and even archeological studies, meteorites have remained a riddle for the human mind. If reason has succeeded over the centuries to make most of their mysteries intelligible, the fascination of these “stones fallen from the sky” nevertheless continues to pose new questions.

The National Aeronautics and Space Administration (NASA) has released the Near Earth Object Program, which tells us that approximately 100 tons of “fairly small natural objects,” mainly rock fragments and dust particles, fall onto the Earth from interplanetary space every day. As they enter the atmosphere at more than 62,000 miles per hour, they heat up and catch fire, forming a luminous trail. They are then called meteors (or shooting stars). Although most meteors burn up completely before they reach the ground, some of them collide with the Earth’s surface. A meteor that does not burn up as it crosses the atmosphere becomes a meteorite. It partly disintegrates on hitting the ground, and its fragments can weigh anything from a few ounces to a couple of tons and spread over several miles. Laboratory, astronomical, and theoretical studies have shown that meteorites are products of collisions that occur within the asteroid belt between approximately 2.1 and 3.3 Astronomical Units (AU) from the Sun (one AU is the average distance from Earth to the Sun, approximately 93 million miles). It is within this region that strong gravitational perturbations by the planets, especially Jupiter, can put meteoroids (the name given to bodies of matter moving in space before they enter Earth’s atmosphere) into Earth-crossing orbits. However, not all meteoroids are formed in the asteroid belt. A few may be the remains of cometary nuclei that originated outside this region, and fewer than 1% of meteorites are recognized to be samples of debris from the surface of the Moon and Mars that have been ejected by impacts.

This presentation is divided into two parts. In the first part, attendees will learn how to identify a meteorite and the phenomenon of the black market in meteorites, which is as organized as any drug trade and just as illegal, will be discussed.

Subsequently, this presentation will present an analysis of 53 meteorites that, from the early 19<sup>th</sup> century to 2018, have hit artificial structures, animals, and people. Although scholars have not been able to confirm the accident nor the fall, on January 16, 1825, scientific memories reported the first “death by meteorite”: a man was killed and a woman injured during a meteorite fall in Oriang, India. Two years later, on February 16, 1827, a man was wounded severely in the arm by an ordinary chondrite meteorite that fell in Mhow, India. On November 11, 1836, chronicles reported the first animal to be killed. It was a cow hit by a shower of meteorites in Macau, Brazil. On August 8, 1863, a 5.4-Kg enstatite chondrite penetrated the roof and floor of a building in Pillistfer, Estonia. On September 15, 2007, a fireball was witnessed landing near Carancas, Peru. Villagers were sickened by the fumes released from an underground water supply tainted with arsenic after the impact. On February 15, 2013, a meteorite exploded above Chelyabinsk, Russia, injuring 1,200 people and causing \$33 million in damage.

In conclusion, particular attention will be devoted to the case of Ann Hodges, who was hit by a meteorite that crashed through the roof of her home and smashed into her hip, leaving a pineapple-shaped bruise, on November 30, 1954, in Sylacauga, AL. The event caused a worldwide media sensation and a year-long legal battle. The meteorite, which weighs approximately 3.85kg, is on permanent display at the Alabama Museum of Natural History.

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### Case Report, Cosmic Forensics, Meteorite



### LW3 The Epic of the World War II (WWII) Italian Submarine *Macallè* and the Death of Carlo Acefalo: How Forensic Science Brought a Soldier's Remains Back Home

Matteo Borrini, PhD\*, Liverpool John Moores University, Liverpool, AE L3 3AF, UNITED KINGDOM

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of the potential and the ethical implications of the forensic anthropological approach on historical cases.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an example of an investigation to recover, identify, and bring back to his home country the remains of a WWII soldier.

In June 1940, the Italian submarine *Macallè* left berth at the naval base in Massawa to hunt for British ships in the Red Sea. Soon after the boat's air conditioning was turned on, a deadly invisible gas, methyl chloride, began leaking throughout the hull. At 02.35 a.m. on June 15, the incapacitated crew led the submarine to collide with the reef of Barra Musa Kebir, a small island 65 nautical miles southeast of Port Sudan. *Macallè* remained trapped between the corals for nearly a day, allowing the crew of 45 men to make it ashore.

The captain sent three men in the submarine dinghy for help in Italian-controlled Eritrea, approximately 200 miles away. With an improvised sail made with shirts and bedsheets, the trio crossed miles of sand bars and marshy coasts; after six days, they were spotted by Italian coastal troops and were able to report the wreck of *Macallè*. Meantime, conditions on the small desert island were steadily deteriorating. The lack of food and water was further weakening the men already poisoned by the methyl chloride. The crew tried without success to distill water and the few fish and birds they were able to catch were insufficient to keep them all alive.

At approximately 3.00 p.m. on June 19, the young Chief Torpedoman, Carlo Acefalo, died of poisoning and starvation and was buried by his comrades under a few inches of sand.

Nearly a week after the sinking of the submarine, the sound of a plane's engine was heard. The joy of an expected rescue was soon tempered when the survivors realized it was a British Royal Air Force aircraft that dropped a note instructing the crew to prepare to be taken to Port Sudan on a Royal Navy vessel. However, a few minutes later, another plane was heard, an Italian one that assured the men that a friendly submarine was on its way to save the castaways. Submarine *Guglielmotti* rescued the crew of *Macallè* just hours before the arrival of British forces, who found only the grave of Carlo Acefalo and the diary of one of the soldiers.

That diary, as well as documents of the "Special Commission of Inquiry into the loss of the *Macallè*", have been used by an Italian-Argentine team to plan a survey in Barra Musa Kebir in October 2014. The underwater photography expedition found metal debris of the sunken submarine 55-60 meters deep in the sea around the island. In addition, on the island, possible clues of Acefalo's grave were found: a circle of stones and rusty fragments. The metal pieces apparently belonged to a Davis auto-respirator, an underwater escape device used by Italian submariners.

After more extensive archival research led to the location of Acefalo's relatives and families of other crew members who provided additional documents, a new scientific expedition was organized in October 2017 with the support of the Sudanese government. The forensic archeological excavation of the burial site was conducted for three days under very difficult environmental conditions. The remains appeared extremely fragmented and damaged due to the characteristics of the grave and the intense heat of the sand under which they had rested for more than 70 years. However, the forensic anthropological examination, as well as the analysis of contextual information and the comparison with historical data, allowed a positive identification of Carlo Acefalo.

The remains of the Italian soldier were turned over to the Sudanese authorities in a ceremony held at Port Sudan on October 9, then consigned to the Italian Ambassador on April 18, 2018, for the final journey home, where Carlo will be buried next to his mother, who died in 1978 awaiting his return.

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#### Forensic Anthropology, WWII Soldiers, Human Identification



### LW4 Three in One Homicide Fire Victims: Dental Identifications the Hard Way

Warren D. Tewes, DDS\*, *Office of the Chief Medical Examiner, State of MD, Queenstown, MD 21658-1101*

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**Learning Overview:** The goal of this presentation is to demonstrate (the principle of) diligence in forensic odontology for dental identification.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by inspiring creative thinking in a seemingly unproductive investigation by reevaluating existing evidence from fresh and different perspectives, while revealing new evidentiary findings by interdisciplinary collaboration.

This principle is presented via related case illustrations and the demonstration of three burned-beyond-recognition fire victims. Diligence, far beyond the usual and customary, is required for this triple homicide scenario because no antemortem dental images or written records existed. More obstacles were present in this 1998 case because modern digital technology was not available to supplement data analysis that is commonplace today. Adding to objectives of diligence is the work ethic of dedicated problem solvers to re-think and create alternative approaches to a seemingly exhausted investigation when conventional methods become fruitless. The diligence becomes contagious for law enforcement and forensic professionals as a spontaneous cohesive devotion evolves and presents as an interdisciplinary team. They guide an effort that is focused and dedicated to the victims' family. Their analysis of the existing available and newly recovered data, in a very timely fashion, leads to the formation of accurate and positive outcomes in grieving circumstances.

The proposition of this presentation is to show that, in the absence of conventional data to perform a more standard human dental identification, with creativity, resourcefulness, collaboration with law enforcement, and scene trace evidence, scientists can yield successful results. The components of this previous statement alone can be considered a composite definition of diligence, dedication, and devotion.

The presentation synopsis is an individualized account of three, burned-beyond-recognition homicide fire victims. While each victim had some teeth missing that could have expedited a positive identification for each, the family reported that they extracted their own teeth. Consequently, no antemortem dental records were ever made and thus were not available for conventional antemortem and postmortem comparisons. Further, in this extraction technique, the teeth were made mobile slowly over weeks using left and right finger pressure movement on the painful tooth. This resulted in no root fractures and minimal trauma to hard and soft tissues, which then facilitated healing.

To conclude, the spirit of diligence, dedication, and devotion, fosters team building across all law enforcement and forensic sciences. This presentation reinforces and encourages an adage of older, experienced members to younger forensic sciences community members, guiding them to "think outside the box."

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**Dental Identification, Alternative Dental Records Search, Forensic Scientists' Collaboration**



### LW5 A Modern Case of Pitt-Hopkins Syndrome With Comparison to a Historic Case of a Feral Child Discovered in the Forest of Germany

Cristin Marie Rolf, MD\*, State of Alaska, Anchorage, AK 99507

**Learning Overview:** The goal of this presentation is to educate attendees about the recognition of a rare congenital disorder characterized by breathing abnormalities with apnea and seizures, which could lead to sudden death.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community through the recognition of a recently described, rare genetic syndrome in a historic case with documented dysmorphic features in hand-rendered pictures, such as a painting, and symptoms in written documentation.

The case was that of a nine-month-old female child with congenital anomalies and mental disability who was referred to the medical examiner as a sudden death under unknown circumstances at her residence. She was born at 34 weeks gestational age via vaginal delivery with intrauterine growth retardation at 1,510 grams and a head circumference of 28 centimeters. Labor was induced due to worsening oligohydramnios. Prenatal ultrasound revealed fetal anomalies including a 2-vessel umbilical cord, situs inversus of the stomach (on right side), cardiac anomalies (interrupted inferior vena cava with continuation through the azygous vein, patent ductus arteriosus, patent foramen ovale and levocardia), hypoplastic corpus callosum and feet positioning abnormalities. Genetics testing revealed chromosomal 18q21.2q23 deletion. Symptoms included periodic breathing with desaturations and severe developmental delay, marked eczema, poor growth, increased sweating, and suspected cortical blindness. At the time of her death, she was known to have had diarrhea for five days. The infant was placed in a bassinette on her right side the evening prior to death and was heard early the next morning making her usual sounds. Later that day, she was found unresponsive, in a supine position with no airway-obstructing materials nearby.

Autopsy revealed an atraumatic, less than third percentile size, mildly dysmorphic female infant. Findings included microcephaly, deeply set eyes, low frontal hairline, curly hair, eczema of the forehead and scalp, prominent lips, situs inversus of the stomach, polysplenia, and appendix. A thin corpus callosum with absence of the cavum septum pellucidum was noted. Gross and microscopic examination of the small and large intestine were unremarkable for pathogenic changes. The toxicology screen was negative. The vitreous electrolytes were significant only for a mildly elevated urea nitrogen of 43mg/dL. The cause of death was attributed to complications of Pitt-Hopkins Syndrome. The mechanism of death most likely originated from abnormalities of breathing with hypoxia or a seizure.

Research in the present case disclosed a historic case of a male child of unknown parentage discovered in the forest of Germany in 1725 by a hunting party of King George I of England. This feral child was uncivilized, walking on all fours, without language skills. When moved to London, attempts to teach him to speak, read, or write were unsuccessful. He had typical features seen in Pitt-Hopkins Syndrome, such as course, curly hair, drooping eyelids, short stature, curvy “Cupid’s bow” lips, and thick lips. After the initial public curiosity, the child was moved to live the remainder of his life on a farm in Northchurch England. Because he would wander off, a leather collar with the inscription “Peter the Wild Man” was placed around his neck. He died around the age of 71 or 72 in 1785 and was buried in Northchurch with a gravestone stating “Peter the Wild Boy.”

Pitt-Hopkins Syndrome is a congenital condition manifesting as moderate to severe intellectual disability with characteristic dysmorphic features and symptoms of breathing abnormalities with apnea and hypoxia. Seizures, delayed psychomotor skills, and stereotypical extremity movements are also seen. Autistic features are also common. The genetic anomaly most commonly seen is a deletion of chromosome 18 (18q21.2q23). The gene affected is Transcription Factor 4, which codes for a protein controlling cell differentiation and apoptosis in the brain, muscles, lungs, and heart. The physical findings seen in a painting and later hand-rendered pictures of Peter and written documentation of his psychomotor disability were recognized as those found in Pitt-Hopkins Syndrome. This is an interesting and educational comparison of a modern case to that of a historical case of a man who beat the odds of surviving for an unknown time alone foraging in the woods with severe disabilities.<sup>1-5</sup>

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#### Pitt-Hopkins Syndrome, Deletion 18q, Haploinsufficiency



### LW6 The Mystery of a 17<sup>th</sup>-Century Epidemic Solved Through Forensic Genealogy

*Colleen M. Fitzpatrick, PhD\*, Fountain Valley, CA 92708*

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**Learning Overview:** After attending this presentation, attendees will understand how multidisciplinary forensic analysis has been used to determine the cause of a devastating epidemic that plagued the residents of Sigolsheim, a village in 17<sup>th</sup>-century Alsace Lorraine, France. The only evidence that an epidemic took place at all was provided by the local church registry of village births, marriages, and deaths, indicating that from 1674 to 1684, nearly every pregnancy in the village resulted in either a miscarriage or stillbirth. Before this, the church records showed that the village had a healthy birth rate and was growing, and that afterward, the population quickly returned to its former level. It was as if a “birth-switch” was abruptly turned off in 1674, only to be turned back on ten years later. The only other telltale clue was a brief increase in death rate in 1664-1665, including the deaths of the entire 11-member Naegler family with the exception of the oldest child, their 18-year-old son, Christopher.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illustrating how much information can be extracted by applying a multidisciplinary approach to very old mysteries where little documentation has survived. While it is exciting to see modern forensic tools applied to modern cases, it can be even more interesting to see these same tools applied to solving old historical cases in a context inconceivable to those who lived so many generations in the past.

Historical reasons for the epidemic are scant. There were no major wars in Europe at that time that would have reduced the birth rate by drawing husbands and sweethearts away from the area for such an extended period. Contaminated water could also be ruled out, as could the possibility of a communicable disease—otherwise the entire village would have perished. Changes in childbirth practices could be eliminated since there were few deaths recorded among women of childbearing age. So, what was the cause of this mysterious epidemic that seem to affect only unborn children, but hardly any other villagers?

The answer might be ergot, a fungus that grows on rye. Ergot has been suspected as the cause of the Bubonic Plague as well as the Salem Witch Trials. It was so common during the Middle Ages that it was thought to be a natural part of the rye plant. Ergot is the original source of LSD.

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**Forensic Genealogy, Epidemic, Multidisciplinary**





## Young Forensic Scientists Forum— 2019

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Y1    WITHDRAWN



## Young Forensic Scientists Forum— 2019

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### Y2 Persistence of Tool Marks on Bones After Burial

Cydnee A. Wolfe\*, Arizona State University, Gilbert, AZ 85296

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**Learning Overview:** After attending this presentation, attendees will have a better understanding of how tool marks persist on bones after burial for varying lengths of time in different substrates and at different depths.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an increased understanding of the reliability of tool mark data taken from bones found in outdoor environments.

When biological remains, such as bones, are part of a crime scene, it is known that biotic and abiotic factors are immediately at work, affecting the presentation of evidence to crime scene investigators. The impact of various biotic and abiotic factors will change over time with increasing exposure of the remains to these factors. In an attempt to quantify the impact of such factors on the reliability of data collected from remains, this study created a controlled experiment in which soil type, burial depth, and length of burial time was varied. This study obtained 36 fresh bovine bone fragments, each approximately 10cm in length, and coarsely cleaned the flesh from the bones, although there was still a significant amount of flesh present on the bones prior to burial. The bones were stored frozen to halt any biotic action prior to placement in the treatments. The bones were “tool marked” using a standard Dremel® blade to create repeatable mark types, but to varying depths and lengths. The depth of the cuts were treated as a continuous variable and ranged from superficial to ~3mm in depth. Each bone was marked three to six times with varying depths of the mark. From each marked bone, a cast was made using Mikrosil™, and the depths of the marks were measured from the casts using a digital caliper. The bone fragments were divided into four bins (nine bones apiece); two bins were filled with store-bought play sand and two were filled with clay-soil obtained from the local environment. The soil type was quantified using standard soil sieve sets. Bones were buried at three depths, top, middle, and bottom. The bins were kept in an outdoor experimental facility with chain-link fencing on all sides, including the top, and were therefore exposed to naturally occurring biotic and abiotic influences, but vertebrate scavengers were effectively excluded. Bones were retrieved at one week, two weeks, and four weeks, starting with the most shallow bones so as not to disturb bones remaining in the treatments. As each bone was recovered from each bin, they were brushed clean, a Mikrosil™ impression was made of the tool marks, and the dimensions of the tool mark casts were again measured with a digital caliper and recorded.

The resulting data indicated a change in the size of tool marks over time, with superficial tool marks decreasing in size and some deeper tool marks appearing to increase in size, with no clear effect of soil type. Time in the bins and depth of burial seemed to amplify this trend, although additional experiments are needed to tease out the different factors more clearly.

*Thank you to the School of Mathematical and Natural Sciences for providing funding for this research as well as the Arizona State University Student Government for travel support.*

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### Tool Marks, Bones, Burial



## Young Forensic Scientists Forum— 2019

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**Y3    WITHDRAWN**



## Young Forensic Scientists Forum— 2019

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### Y4 Analysis of Upconverting Nanoparticles for Latent Fingerprint Detection

*Hannah K. Weppner\**, Rochester Institute of Technology, Rochester, NY 14623; *Sierra R. Rasmussen, BS*, South Dakota School of Mines & Technology, Rapid City, SD 57701; *Jon Kellar, PhD*, South Dakota School of Mines and Technology, Rapid City, SD 57701; *William Cross, PhD*, South Dakota School of Mines and Technology, Rapid City, SD 57701

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**Learning Overview:** The goal of this presentation is to present research conducted with the Security Printing and Anti-Counterfeiting Research Experience for Undergraduates (REU) at the South Dakota School of Mines and Technology. The research focused on testing and optimizing applications of upconverting nanoparticles to improve latent fingerprint detection on difficult substrates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing an investigation into a potential latent fingerprint detection method that, if fully optimized and implemented, could improve the detection of latent fingerprints on fluorescent substrates. This presentation details current application methods, analyzes the utility of alternative application methods, and suggests the direction of future research.

Because of fingerprints' value as forensic evidence in courtrooms around the world, continued research into latent fingerprint development methods remains important. In this study, two novel application methods for NaYF<sub>4</sub>:Yb,Tm Upconverting Nanoparticles (UCNPs) are considered. UCNPs have previously been investigated for their utility as latent fingerprint-detecting powders given their anti-Stokes properties that can allow for the visualization of fingerprints on colorful, fluorescent backgrounds, which are currently incompatible with many traditional development techniques. Donor fingerprints were deposited on cleaned glass and silicon substrates using a split print method to directly compare the effects of two methods. The UCNPs were prepared in organic solvent dispersions of toluene and chloroform and a soybean oil-in-water emulsion and applied by immersing the substrate, and, in the case of the emulsion, spraying the substrate. The results were subsequently imaged using a 980nm laser and camera, a Scanning Electron Microscope (SEM), an optical profilometer, and a video spectral comparator (VSC).

The UCNPs emulsion was found to have little utility as a fingerprint development agent because of the oil residue left on substrate. This residue was difficult to remove and impaired imaging. The toluene dispersion appeared more promising, especially when prepared in a concentration of 1wt% and when washed by immersing in toluene following the application of the dispersion. Additionally, the fingerprints did not appear to dissolve in the toluene when submerged for short periods. With this application and proper imaging, visualization of fingerprint ridges was possible on some of the glass and silicon substrates tested. Further research should concentrate on the formulation and application of organic solvent dispersions to improve the adhesion to the print, reduce background staining, and improve imaging procedures. Additionally, investigating upconverting nanoparticles of varying sizes may also optimize imaging.

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### Nanoparticles, Latent Fingerprints, Upconversion



## Young Forensic Scientists Forum— 2019

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### **Y5 A Hemorrhage of Cerebellum and Brainstem Due to Cervical Epidural Steroid Injection: A Case Report and Review of Literature**

*Connie Bao\**, Champaign County Coroner's Office, Urbana, IL 61802

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**THIS ABSTRACT WAS NOT PRESENTED.**



## Young Forensic Scientists Forum— 2019

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### Y6 Using Cell-Free DNA to Improve Short Tandem Repeat (STR) Analysis of Sweat Samples

*Nathan R. McFadden\*, Pittsburgh, PA 15203*

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**Learning Overview:** After attending this presentation, attendees will learn how cell-free DNA may be a suitable source of DNA from sweat samples to improve upon STR profiles of low copy number DNA.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by improving upon low copy number samples, such as touch DNA (tDNA), through the use of cell-free DNA.

In the forensic community, tDNA samples are known to be difficult samples to obtain an ample amount of template DNA and produce DNA profiles using STR analysis. Cell-free DNA (cfDNA) is DNA present outside the cell and is found in bodily fluids, such as sweat, saliva, and blood serum. The use of cfDNA has typically been for cancer and prenatal diagnoses of a mother and her child. However, previous research has thought cfDNA could be used to enhance DNA profiles obtained from tDNA samples. For forensic purposes, extractions have not been conducted with a cfDNA extraction kit.

In the first phase of this study, QIAGEN® extraction kits were used to extract DNA from blood serum samples. The two QIAGEN® kits used were the QIAamp® DNA Mini Kit and the QIAamp® Circulating Nucleic Acid Kit. The Circulating Nucleic Acid Kit uses vacuum filtration through a silica membrane with different lysis and wash buffers than the DNA Mini Kit. DNA yields and STR profiles were compared between the two kits and confirmed the Circulating Nucleic Acid kit provided larger amounts of extracted DNA and improved profiles.

In the second phase, sweat samples were collected on glass beads, with a diameter of 0.5cm, then extracted with the Circulating Nucleic Acid Kit. The samples were quantified with Quantifiler™ and genotyped with Promega® Fusion 6C to validate the use of cfDNA for forensic use. Quantitation values found in the second phase were consistent with the quantitation values found in the first phase, using blood serum. STR profiles generated from the extracted and amplified samples showed signs of major and minor contributors. Although Relative Fluorescence Units (RFU) values were small, there were prominent peaks and smaller peaks present in some of the electropherograms. The possible source the minor contributor could be attributed to the handling of items and interactions with others throughout the day.

The third phase of this research will include a larger sample size and will look to find the source of DNA by quantifying and genotyping both the cell pellets and supernatant from the extraction of each sweat sample. If cfDNA can be validated using sweat samples, cfDNA could be a key component in the genotyping of touch DNA samples. The forensic use of cell-free DNA still needs to be validated further, but if quantities and STR profiles of low template number DNA can be consistently reliable using cfDNA, it may lead to stronger evidence and further the use of DNA genotyping.

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#### Cell-Free DNA, Short Tandem Repeat Profiles, Low Copy Number



## Young Forensic Scientists Forum— 2019

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### Y7 Detection of Undeclared Substances and Heavy Metals in Alternative Medicines by Chemical Analysis

*Nicole Almeter, BS\*, Arcadia University, Glenside, PA 19038; Jonathan Orr, Arcadia University, Glenside, PA 19038; Karen S. Scott, PhD, Arcadia University, Glenside, PA 19038; Shanán S. Tobe, PhD, Arcadia University, Glenside, PA 19038; Heather L. Harris, JD, Glenside, PA 19038*

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**Learning Overview:** After attending this presentation, attendees will understand the possible dangers of herbal medicines that can be legally purchased in the United States. Attendees will recognize useful presumptive and confirmatory tests that can be used to characterize these medicines and to identify possible heavy metals and undeclared drugs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a variety of analytical techniques that can be used to characterize unknown substances within complex matrices.

Modern herbal medicines based on traditional Chinese medicines have become an increasingly common choice for consumers to treat many ailments from backaches to allergies to migraines. Many people choose these medicines because they believe they are safer alternatives to, or contain more natural ingredients than, western pharmaceuticals. However, recent reports in scientific literature have indicated possible heavy metal poisoning or adverse effects from undeclared substances present in herbal medicines. Despite these case reports, herbal medicines, like other dietary supplements, are unregulated by the Food and Drug Administration (FDA). Herbal medicines are forensically relevant because undeclared substances may appear in toxicological analysis or these medicines may be found at a crime scene and analyzed by a forensic chemist. Thus, more research is needed to characterize possibly adulterated herbal medicines.

This research aims to identify heavy metals, including arsenic, lead, and mercury and/or organic adulterants, including undeclared drugs, in herbal medicines through various analytical techniques that can be implemented in a forensic laboratory. Ten different herbal medicines have been purchased from traditional Chinese medicine shops in different cities in the United States, including Seattle, WA, New York City, NY, and Philadelphia, PA. Presumptive tests, such as polarized light microscopy, Fourier Transform Infrared Spectroscopy (FTIR), and color tests have been performed. These tests are important because they can guide the analysis of herbal medicines that often contain unknown substances and have complex matrices.

Polarized light microscopy was found useful to identify fillers in these medicines, including sand and starch. Both transmission and Attenuated Total Reflectance (ATR) sampling were used with FTIR in order to indicate possible adulterants, including warfarin, dextromethorphan, and netilmicin, as well as provide information on major components of the medicines. Color tests were used to test for heavy metals and possible drugs. Using the Reinsch test, four samples had a positive indication for arsenic and one sample had a positive indication of mercury. Using Mandelin's reagent, one sample had a positive indication of ibuprofen. From the results of these tests, future work can include analysis by Atomic Absorption (AA) spectroscopy and Gas Chromatography/Mass Spectroscopy (GC/MS).

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#### Herbal Medicines, Undeclared Substances, Heavy Metals



## Young Forensic Scientists Forum— 2019

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### Y8 The Analysis of Fentanyl Analogs by Gas Chromatography/Solid State Infrared Spectroscopy (GC/IR)

*Emily Prisaznik, MS\*, 2815 W Highland Street, Allentown, PA 18104; Thomas A. Brettell, PhD, Cedar Crest College, Allentown, PA 18104*

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**Learning Overview:** After attending this presentation, attendees will be introduced to a new method that meets the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) guidelines for the analysis of fentanyl and its analogs.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by allowing forensic labs and scientists to utilize a new method for the analysis of fentanyl and its increasing analogs.

An investigation of the analysis of fentanyl and ten fentanyl analogs by GC/IR has been performed. GC was performed using an Agilent® 6890N gas chromatograph. IR spectroscopy was performed on a Spectra-Analysis® DiscovIR-GC® using an operation system with Thermo™ Galactic™ GRAMS 8.0. The cryofocused disk was cooled with liquid nitrogen to -40°C and the dewar was set to 20°C for all experiments. GC/solid-state IR spectroscopy has shown to be capable of identification of fentanyl and ten of its analogues (4-ANPP, acetyl fentanyl, acryl fentanyl, alfentanyl HCl, butyryl fentanyl, cyclopropyl fentanyl, crotonyl fentanyl, furanyl fentanyl, isobutyryl fentanyl, valeryl fentanyl). The method, using the conditions in this study, is capable of producing a reproducible quality solid-state IR spectrum from injection of 1µL of a 1mg/mL methanolic solution of sample onto a chromatographic column. Using the chromatographic conditions in the method developed, most of the fentanyl analogues can be separated such that IR spectra can be produced for identification of the compound. Most of the fentanyl analogues were separated with 99% confidence ( $s=3sd.$ ) except for three pairs, isobutyryl fentanyl/fentanyl, fentanyl/acryl fentanyl, and butyryl fentanyl/cyclopropyl fentanyl. Multiple injections of the fentanyl compounds resulted in reproducible retention times (standard deviation=0.023 and a CV% of 0.012 %). The data also produced spectra containing absorption peaks with an average standard deviation=0.272 and an average CV%=0.022, showing good reproducibility for the IR spectra. The solid-state IR spectra of fentanyl and the ten fentanyl analogues analyzed in this study were quite similar but were easily discriminated by comparison of the fingerprint region of the spectrum. The amide region ( $1,650\text{cm}^{-1}$ ) clearly showed different absorption peaks and pattern as well as the region for the C-H stretches of the aromatic and alkyl region ( $2,700\text{cm}^{-1}$ – $3,050\text{cm}^{-1}$ ) and the spectral region between  $1,200\text{cm}^{-1}$  and  $1,500\text{cm}^{-1}$ . The linear retention indices for the fentanyl compounds were calculated using a qualitative retention index standard mixture.

Overall, GC/solid-state IR spectroscopy is a viable and reproducible method for the analysis and identification of fentanyl and fentanyl analogues that may be found in seized drug material.

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#### Fentanyl, Gas Chromatography, Infrared Spectroscopy





## Young Forensic Scientists Forum— 2019

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### **Y9 Are Intra-Alveolar Hemorrhage and Aspiration of Bacterial Colonies Pathognomonic in Asphyxia by Suffocation in Infancy?**

*Cara A. Mitrano\*, Midland, MI 48642; L.J. Dragovic, MD, Oakland County Medical Examiner's Office, Pontiac, MI 48341; Andrew Hanosh, MD, Oakland County Medical Examiner's Office, Pontiac, MI 48341; Bernardino Pacris, MD, Oakland County Medical Examiner's Office, Pontiac, MI 48341; Ruben Ortiz-Reyes, MD, Oakland County Medical Examiner's Office, Pontiac, MI 48341; Kanubhai P. Virani, MD, Oakland County Medical Examiner's Office, Pontiac, MI 48341*

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**Learning Overview:** After attending this presentation, attendees will understand: (1) histological findings present in cases of infant death due to asphyxia by suffocation, (2) the mechanism of death that supports these findings, (3) key techniques in slide analysis, and (4) how to apply these lessons in the context of death investigation.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by illuminating a relationship between intra-alveolar hemorrhage, freshly aspirated bacterial colonies, and infant death due to asphyxia by suffocation. The distinction is critical in medicolegal death investigation that is case-specific. When an infant is discovered unresponsive, it is typically rushed to the nearest emergency room facility, resulting in a disturbed scene of death. Physical findings documented at the scene are compared with those documented at the time of autopsy. The rather randomly reported microscopic findings of sub-pleural and intra-alveolar hemorrhage and freshly aspirated bacterial colonies in the alveolar space (without signs of inflammatory response) are useful in diagnosing asphyxia by suffocation. Results of this study provide an additional diagnostic tool that improves the accuracy of investigation and determination of cause and mechanism of death.

The purpose of this study was to examine intra-alveolar hemorrhage and freshly aspirated bacterial colonies as possible pathognomonic findings in cases of infant asphyxia by suffocation. To study this relationship, a review of autopsy materials (1970–2017) archived at the Oakland County Medical Examiner's Office (OCMEO) was conducted and revealed 1,161 infant deaths. Available histological slides were examined independently by multiple pathologists for evidence of intra-alveolar hemorrhage and freshly aspirated bacterial colonies. The independent review by multiple parties was necessary to determine the statistical significance of these findings in cases of infant death due to asphyxia by suffocation. The year 1992, when mandatory thorough death scene investigation was fully implemented in Oakland County, led to discontinuation of the diagnosis of Sudden Infant Death Syndrome (SIDS). This energized efforts in prevention of infant deaths through implementation of safe sleep environments and detailed documentation of evidence at the scene of death using re-enactment. In these cases, histological findings with consensus among reviewers were compared to autopsy reports and notes from scene investigations. This provided full context of each case. Cases of infant asphyxia were then compared to infant deaths due to other causes as controls.

Preliminary findings indicate that intra-alveolar hemorrhage is present in most cases of infants suspected of dying from asphyxia by suffocation. Freshly aspirated bacterial colonies are frequently accompanying, though somewhat less common. No significant morphologic difference has been observed in cases of accidental vs. homicidal asphyxia by suffocation. These findings provide additional information to pathologists and death investigators seeking clarification in cases of infant death due to asphyxia by suffocation, especially in the absence of evidence supporting another cause of death. In conclusion, intra-alveolar hemorrhage and freshly aspirated bacterial colonies are useful in determining cause of infant deaths due to asphyxia by suffocation.

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#### **Infant Death, Asphyxia, Intra-Aleveolar Hemorrhage**



## Young Forensic Scientists Forum— 2019

### Y10 Comparison of Periodic Acid-Schiff Staining With Lugol's Iodine for the Detection of Vaginal Fluid in Dried Material

Melissa Rogers\*, North Wales, PA 19454; Lawrence Quarino, PhD, Cedar Crest College, Allentown, PA 18104

**Learning Overview:** The goal of this presentation is to illustrate the stark differences observed between vaginal fluid, saliva, and urine when stained with the Periodic Acid-Schiff (PAS) stain.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing the foundation for a potential confirmatory test for vaginal fluid, and so provide context for sexual assault investigations.

Forensic science literature presents a variety of tests to confirm the presence of various bodily fluids; however, such a test for vaginal fluid is still needed. A confirmatory test for vaginal fluid will provide critical context for forensic casework, especially in sexual assault cases in which no semen is present. In the past, Lugol's iodine was used as the main method for determining the presence of vaginal fluid by staining glycogen.<sup>1</sup> However, this type of staining has been shown in the present study to produce positive results for both saliva and urine, demonstrating its lack of specificity. Although not frequently used as a test for the presence of vaginal fluid, the PAS reagent has been reported to be effective in staining epithelial cells high in glycogen.<sup>2</sup> This study aims to compare the ability of these two stains to detect the presence of vaginal fluid in dried material.

Following Institutional Review Board (IRB) approval, multiple vaginal swabs, saliva swabs, and urine samples were obtained from female participants who varied in age, menstrual cycle, and birth control methods. The first stage of this study involved extracting the glycogen from these samples, then adding a 3% solution of Lugol's iodine to each extract to sample wells in a microtiter plate. The absorbance of each sample was then measured with subsequent quantitative analysis for glycogen using a standard curve. The results of this method indicated that the concentration of glycogen in urine was very similar to that of vaginal fluid, and so could not be distinguished from each other. Concentration values in saliva were also not appreciably lower. Staining of vaginal smears on microscope slides with Lugol's iodine also did not show observable differences between the three fluids. Conversely, for the second stage, vaginal smears showed significant observable differences in glycogen staining with PAS when compared to smears made from saliva and urine. Differences were so pronounced that absorbance assays would simply be redundant. Results between women of varying age were comparable.

Not only do these results bring to light a more effective stain for vaginal fluid, but also provides the foundation for a potential confirmatory test that could be used in serology laboratories. Development of such a test for vaginal fluid will help provide context for sexual assault investigations.

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1. Rothwell T.J., Harvey K.J. The Limitations of the Lugol's Iodine Staining Technique for the Identification of Vaginal Epithelial Cells. *J Forens Sci Soc* 1978 Jan;18:181-184.
2. Jones E.L. The Identification of Semen and Other Body Fluids. In: Saferstein R, editor. *Forensic Science Handbook: Volume II*. Upper Saddle River, 2005;360-68.

#### Serology, Vaginal Fluid, Periodic Acid-Schiff



## Young Forensic Scientists Forum— 2019

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### Y11 The Analysis of Fentanyl and Its Analogs and Metabolites in Postmortem Blood Using Biocompatible Solid-Phase Microextraction (BioSPME) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Gabriella Smith, BS\*, Cedar Crest College, Schnecksville, PA 18078; Thomas A. Brettell, PhD, Cedar Crest College, Allentown, PA 18104; Chandler Marie Grant, MS, Allentown, PA 18106; Marianne E. Staretz, PhD, Cedar Crest College, Allentown, PA 18104; Thomas H. Pritchett, MS, Cedar Crest College, Allentown, PA 18104

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**Learning Overview:** After attending this presentation, attendees will be informed on a new possible analysis method for fentanyl and its analogs using a BioSPME extraction and LC-MS/MS that decreases analysis time.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a newly validated method for an efficient extraction and overall analysis of fentanyl and its analogs in the height of the opioid epidemic.

In 2016, approximately 13 people died of a drug-related overdose each day in Pennsylvania. In all, 4,642 drug-related overdose deaths were reported that year by Pennsylvania coroners and medical examiners. In 52% of these cases, fentanyl and fentanyl-related substances were identified in decedents, with heroin being the second most frequently identified substance. This is an increase of 27% from the previous year. Due to the ease with which these illicitly manufactured fentanyl and fentanyl analogs can be synthesized, it is essential that new validated methods be investigated. In addition, it is often difficult to synthesize standard reference materials for the increasing number of illicitly manufactured fentanyl analogs. It is also expensive for laboratories to purchase all the standards needed for developing methods and analyzing samples with these compounds included in the panel. Structural similarities among many of these analogs also present unique challenges for toxicology laboratories. For example, by adjusting the position of a single additional methyl group, a total of 15 different analogs can be synthesized. The presence of fentanyl analog isomers and isobaric compounds causes great difficulty in separation using LC. Additionally, the fentanyl epidemic is also causing an increase in casework. Therefore, the development of more efficient techniques for screening postmortem samples for fentanyl and its analogs would greatly benefit toxicology laboratories.

The purpose of this study is to compare a new *in vivo* BioSPME technique to an existing technique used in the toxicology department at Health Network Laboratories (HNL) located in Allentown, PA. The toxicology department at HNL is a full-service toxicology laboratory, providing therapeutic drug monitoring, compliance drug testing, workplace drug testing, Driving Under the Influence (DUI)/Driving Under the Influence of Drugs (DUID), and postmortem toxicological services. BioSPME was developed as an extraction method that could quickly extract drugs from biological fluids without the binding of macromolecules, which was a concern for previous SPME techniques. The use of BioSPME fibers allows for the direct analysis of a free analyte fraction on traditional Liquid Chromatography/Mass Spectroscopy (LC/MS) methods. These tips also open the door to the possibility of future direct MS analysis that could greatly impact the field by providing a rapid detection method for these fentanyl compounds, a capability that could drastically reduce sample preparation and analysis times.

In this study, a method to analyze 19 different fentanyl analogs in postmortem blood is being developed using both BioSPME and HNL's extraction method. The method will utilize both Time-Of-Flight/Mass Spectrometry (TOF/MS) as a screening method and LC-MS/MS as a quantitative method.

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#### Fentanyl, Toxicology, LC-MS/MS



## Young Forensic Scientists Forum— 2019

### Y12 Optimization of a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Method for the Separation and Identification of Synthetic Cannabinoids on Prison Mail Samples

Alyssa Hurd, BA\*, Harrisburg, PA 17112; Stephanie J. Wetzel, PhD, Duquesne University, Pittsburgh, PA 15282

**Learning Overview:** After attending this presentation, attendees will understand the importance of developing analytical methods to better screen for drug-soaked paper disguised as mail samples being sent into prisons across the country and will understand why there remains a need for established research to ensure that drug-soaked prison mail samples do not pose a further threat to prison staff and inmates.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by describing the method development and validation results using synthetic cannabinoids JWH-018, JWH-073, AM2201, XLR-11, AB-CHMINACA, 5F-ADB, and AM-2233, the most commonly seen as published in the National Forensic Laboratory Information System (NFLIS) Special Report on Synthetic Cannabinoids and Cathinones.<sup>1</sup>

Synthetic drugs, colloquially named with street names such as K2 or Spice, have seen a surge in popularity over the past decade. This popularity has posed a number of problems within the prison system, including the difficulty of detecting drugs coming in through the mail as well as the safety risk it poses to both inmates and prison staff. While numerous published methods exist for human drug screenings after the inmates consume the synthetic drugs, there remains the need for an established method to screen for the drug-soaked mail samples as they are being sent into the prisons.

In this study, a reliable and valid LC-MS/MS method was developed for separating and identifying synthetic cannabinoids utilizing seven synthetic drug standards: JWH-018, JWH-073, AM2201, XLR-11, AB-CHMINACA, 5F-ADB, and AM-2233. All seven drug standards were soaked into white printer paper in equal ratios and used as samples for method development. Matrix effects were accounted for using commonly encountered mail contaminant, such as pen, crayon, and lipstick, due to the unpredictable nature of prison mail. Ultrasonification was utilized to extract the synthetic cannabinoid from the paper substrate, followed by the development of an LC-MS/MS method by varying solvent ratios and optimizing mass spectral parameters. The developed method was then validated by analyzing 47 actual mail samples recovered from the Allegheny County Jail to test the ability of the method in analyzing true synthetic cannabinoid-soaked paper. The findings of this study suggest that a valid and reliable method can be developed, given the variety of synthetic cannabinoid classes tested. Matrix effects were not observed with the tested contaminants, suggesting that the extraction method is sufficient in removing extraneous substances other than the analyte in question.

#### Reference(s):

- <sup>1</sup> Strom, K., Moore, K., Weimer, B., Heller, D., Ancheta, J., Pressley, D., and Polk, A. (2014). *National Forensic Laboratory Information System special report: Synthetic cannabinoids and synthetic cathinones reported in NFLIS, 2010-2013*. U.S. Drug Enforcement Administration.

**Synthetic Cannabinoids, LC-MS/MS, Prison Mail**



## Young Forensic Scientists Forum— 2019

### Y13 The Effects of Household Cleaners and Paints on the Detection of Bloodstains Over Time

Katelin Vietri, BA\*, Mesa, AZ; Maria Cruz Blas, Arizona State University, Glendale, AZ 85306; Kimberly S. Kobjek, MS, Arizona State University New College, Phoenix, AZ 85069-7100

**Learning Overview:** After attending this presentation, attendees will better understand the potential effects on bloodstains from household cleaning products and paint products used when attempting to alter or eliminate bloodstain evidence at a crime scene.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by: (1) identifying potential methods that criminals may use to hide or cover up bloodstains; and (2) illustrating potential challenges with the current methods of bloodstain detection.

Blood and bloodstain patterns are often valuable sources of evidence found at crime scenes. It may be found that the suspect or suspects in the case have attempted to clean up the blood evidence in an attempt to destroy any probative value of such stains. Because of these attempts at clean up, it is important for crime scene specialists and investigators to be aware of the potential challenges that may be faced with collecting or analyzing blood evidence at such a crime scene.

Kastle-Meyer (a phenolphthalein test) has been used for more than 100 years as a sensitive method for the detection of possible blood.<sup>1</sup> Chemiluminescent reagents may be used in conjunction with Kastle-Meyer in an effort to detect “invisible” blood, to include blood or bloodstain evidence in which an attempt has been made to alter or destroy it. Despite the sensitivity of the Kastle-Meyer test and chemiluminescent reagents, there may be unforeseen challenges facing those who process the crime scene and collect bloodstain evidence.

Previous research has demonstrated both that the physical chemiluminescence properties of bloodstains can be altered by cleaning products and that paint will affect the detection of bloodstains; however, research on the effects of both the cleaning and concealment of bloodstain evidence is scarce.<sup>2-4</sup> Additionally, there seems to be a lack of applied research regarding the effects of clean-up on bloodstains; no previous study has discussed whether a bloodstain can be covered up and rendered undetectable by traditional forensic means. A grant awarded to Arizona State University through the National Science Foundation focusing on the implementation of a course-based undergraduate research experience allowed for the development and execution of a research project that would address the lack of applied research in this area.

Blood was applied to a substrate that mimics a substrate that is commonly found at crime scenes and was left to dry. Each bloodstain was subjected to a different combination of clean-up and cover-up procedures. Following these procedures, two common forensic blood indicator tests were used. The results of both the controls and the experimental bloodstains yielded surprising results despite the sensitivity of both the Kastle-Meyer and chemiluminescent reagents, regardless of the clean-up and concealment that took place. The implication of both sets of results could manifest differences in the method for which a crime scene may be processed; investigators could derive helpful information from concealed or covered-up bloodstains, but only if they are aware of the presence of such evidence.

This material is based upon work supported by a National Science Foundation grant. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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#### Bloodstains, Chemiluminescence, Household Cleaners



## Young Forensic Scientists Forum— 2019

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### Y14 Analysis of Drugs Used in Crimes Using Solid Phase Extraction (SPE) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Kimberly E. LaGatta, BS\*, 15 Union Road, Oswego, NY 13126; Vadoud Niri, PhD, State University of New York Oswego, Oswego, NY 13126; Shokouh Haddadi, PhD, State University of New York Oswego, Oswego, NY 13126

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**Learning Overview:** The goal of this presentation is for attendees to learn of an alternative to detecting drugs in very low concentrations that are used in the facilitation of crime.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community as this project aims to develop a more efficient and sensitive method to detect drugs that are commonly used to facilitate crime. The optimized method in this project has lower Limits Of Detection (LOD) than methods currently used in forensic laboratories.

Benzodiazepines are a class of antianxiety drugs that are also known as predator or date-rape drugs used in Drug-Facilitated Crimes (DFCs)—crimes that include robbery and the maltreatment of the elderly and children, as well as rape and other sexual assaults. Identification of these drugs or their metabolites in biological specimens such as urine, blood, saliva and hair of victims is commonly proof of exposure to the drug.

Like other psychoactive drugs used in DFCs, benzodiazepines are highly potent and have short half-lives. The combination of potency and a short half-life makes the time window for when the drug is still detectable in biological samples very small. Victims of DFCs usually experience short-term memory loss and often report the crime days after they happened, which adds to the challenge of detecting the drugs in the biological samples by the currently used analytical methods in forensic toxicology laboratories. The goal of the current project is to develop a method for the detection, identification, and quantification of benzodiazepines, including 7-aminoflunitrazepam, alpha-hydroxyalprazolam, alprazolam, bromazepam, chlordiazepoxide, clonazepam, diazepam, flunitrazepam, lorazepam, and nitrazepam, at very low concentrations in aqueous solutions, which can be adopted for the analysis of these drugs and their metabolites in biological samples such as urine and blood.

An SPE coupled to an LC-MS/MS method was successfully optimized. A Supel™- Select HLB 54183-U 200mg cartridge was selected for SPE. Washing and condition of the packing was completed using 12mL of methanol and 2mL of ultra-pure water, respectively. After sample introduction, the analytes of interest were eluted using 5mL of a 50:50 mixture of methanol and acetonitrile. The elutant was evaporated using rotary evaporation and, upon dryness, was reconstituted using 1mL of 0.01M ammonium acetate. The reconstituted sample was then subjected to LC using a C18 HPLC column (particle size: 1.9µm; length: 20cm) with gradient elution with a mixture of acetonitrile and 0.01M ammonium acetate buffer solution. The elution started with 100% 0.01M ammonium acetate and was gradually decreased to 10%, while acetonitrile was increased to 90% and held for one-half minute. Then 0.01M ammonium acetate was increased back to 100% and held for 4min. For mass spectrometry detection, a Selected Ion Monitoring (SIM) method was started at minute 3.00 of analysis until minute 8.00. Using the optimized SPE and LC-MS/MS method, this study achieved the LOD for ten analytes and their metabolites to be in the range of pg/mL to ng/mL levels, which is much lower than LODs for the common methods used in forensic labs.

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**DFC, Liquid Chromatography, Solid Phase Extraction**



## Young Forensic Scientists Forum— 2019

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### Y15 Fingerprints and Ancestry: Is It All in the Details?

Jessica R. Ford\*, Oklahoma City Police Department, Oklahoma City, OK 73114

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**Learning Overview:** The goal of this presentation is to discuss the plausibility of using Galton features, specifically the types, locations, and number of minutiae, to determine a person's ancestry.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by creating a greater understanding of criminalistics, specifically as it relates to the viability of fingerprints left at a crime scene.

This concept of ancestry from fingerprint minutiae has been explored over the years by Sir Francis Galton, and more recently, Fournier and Ross.<sup>1,2</sup> Galton studied and compared the fingerprints of English pure Welsh, Hebrew, Negro, and some Basques from Cambo in the French Pyrenees to look for differences in patterns and minutiae characteristics<sup>2</sup>. His study had a sample size of more than 100 individuals. While his study did not yield significant scientific results, Fournier and Ross conducted a similar study with the purpose of exploring the influence of sex, ancestry, and pattern type on minutiae in African descendent and European descendent males and females.<sup>1</sup> Overall, 243 right index fingerprints were chosen to include 61 African American females and 61 African American males for a total 122 African Americans as well as 60 European American males and 61 European American females for a total of 121 European Americans. Based on this study, fingerprint minutiae, specifically the total number of bifurcations, show promise as a method to predict the ancestry of an individual to some degree of certainty.

Researchers at the University of Central Oklahoma have been conducting a study made up of 250 participants: 25 Hispanic descendant males, 25 Hispanic descendant females, 25 Asian descendant males, 25 Asian descendant females, 25 Native American descendant males, 25 Native American descendant females, 25 African descendant males, 25 African descendant females, 25 European descendant males, and 25 European descendant females. The sex and ancestry of each participant is ascertained based on self-identification and demographic information. In order to collect the prints, each participant had their right index finger rolled on a ten-print card using fingerprint ink. Each print shows a complete nail-to-nail roll to be recorded for later analysis of friction ridge detail. Because of the statistical likelihood that the right index finger is the print most often encountered at crime scenes, those prints were used for analysis. With the assistance of AFIX Tracker<sup>®</sup> technology, each print was analyzed and marked by the researcher for each of the five main fingerprint minutiae characteristics: bifurcations, enclosures, dots, ending ridges, and short ridges. It has been found that the amount of bifurcations in one's fingerprint yields significant results as relates to ancestry. Interestingly, it has been found that African American descendants have slightly more bifurcations than European American descendants.

Significant results in the above research yields new possibilities for law enforcement personnel. Deriving someone's ancestry from their fingerprints can serve as corroborative evidence that could aid in the conviction of criminals who could otherwise walk free. This presentation could greatly impact the forensic science community by shedding new light on innovative ways fingerprints can be used in the application of criminalistics to crime scenes.

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**Fingerprints, Minutiae, Ancestry**



## Young Forensic Scientists Forum— 2019

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### Y16 Accuracy of Ammunition With the Addition of Luminescent Markers

Jared E. McAtee\*, Renfrew, PA 16053

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**Learning Overview:** The goal of this presentation is to educate attendees on the possible benefits of using luminescent markers in gunpowder for Gunshot Residue (GSR) detection, as well as explain how they affect the accuracy of ammunition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by potentially allowing GSR analysis to be conducted in a timely manner, as well as limiting false positive and false negative results from the Scanning Electron Microscope coupled with Energy Dispersive X-ray Spectroscopy (SEM/EDS), without affecting the accuracy of ammunition

When firearms are discharged, this creates a plume of particles known as GSR composed primarily of lead, barium, and antimony. This residue can be collected and analyzed from a person's hands, face, or the clothing that they were wearing when the firearm was discharged. GSR is commonly analyzed using SEM/EDS, but can also be analyzed using other technologies, such as Raman spectroscopy. The downside to SEM/EDS is the length of time it takes to analyze samples, as well as becoming less robust as manufacturers are ceasing to use lead in their ammunition. The addition of luminescent markers within the gunpowder has been shown to be a viable technique to enhance the collection of GSR, while also having the potential to eliminate collection of non-GSR samples, by using an Ultraviolet (UV) light.

The accuracy of ammunition with the addition of luminescent markers was tested on three different firearms: a .222 rifle, a 30-06 rifle, and a .357 revolver, each at their optimized range (50 yards, 100 yards, and 25 yards, respectively). Ammunition was hand re-loaded while adding luminescent markers in varying weight percentages to the gunpowder that was used in each specific firearm, including 2%, 4%, and 6%. Five samples were loaded in each of the different groups, including a control group in which no luminescent markers were added. After the first and fifth shot in each trial, the hands were observed under a UV light where some particles could be observed. A *t*-test concluded that there was no significant difference between the accuracy of the control groups for each firearm and any of the samples with the luminescent markers. A second trial was performed by removing gunpowder equal to the amount of luminescent powder that was added to ensure that the overall amount of powder remained the same within each cartridge. The same procedure was performed as in the first trial. The results showed at low amounts of luminescent markers replacing gunpowder (98%:2% and 96%:4%), there was no significant difference in the accuracy when a *t*-test was performed comparing them to the control. As more gunpowder was replaced with luminescent markers, the accuracy became significantly worse than the control. Velocities were tested for a sample in each trial at Peterson Cartridge in Warrendale, PA, to see the effects of the luminescent powders. It was found that luminescent markers did affect the velocity for the .222 rifle and 30-06 rifle, but not the .357 revolver.

Production of luminescent markers with a higher temperature resistance is needed for further trials to optimize the ratio of gunpowder to luminescent markers. If it is possible to create ammunition that contains luminescent powders and not significantly affect accuracy, this could greatly increase the potential for GSR analysis being conducted in a timely manner, as well as help limit false negative or false positive results from the SEM/EDS.

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#### Gunshot Residue, Luminescent Markers, Accuracy





## Young Forensic Scientists Forum— 2019

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### **Y17 A Comparison of Sol-Gel Adsorption Phases and Their Extraction Efficiencies of Explosives Using Closed Headspace Sampling Coupled With Capillary Microextraction of Volatiles (CMV)**

*Amy Salyards\*, Sharpsburg, GA 30277; Michelle N. Torres, BS, Florida International University, Miami, FL 33199; Kelsey Patterson, Allentown, PA 18106; Jose R. Almirall, PhD, Florida International University, Miami, FL 33199*

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**Learning Overview:** After attending this presentation, attendees will better understand how changing the sol-gel chemistry of a CMV device can alter the percent recoveries of explosive compounds.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by promoting a faster, more sensitive sampling technique to analyze explosive compounds.

When analyzing explosive residues from a crime scene, one common sampling technique for the detection and extraction of volatile explosive compounds involves headspace sampling, including the use of Solid Phase Microextraction (SPME). Although SPME sampling and extraction is sensitive, CMV, a faster and potentially more sensitive sampling technique is proposed as an improvement over the equilibrium-based SPME extraction. SPME is considered a passive air sampling technique, while CMV is an active air sampling technique; this makes CMV extraction have more mass transfer and reduces sampling time. Previous laboratory studies incorporated a trimethoxymethylsilane sol-gel adsorption phase within the CMV sampler (CMV-A) to analyze low nanogram quantities of volatile explosives. The overall goal of this current effort is to create a new sol-gel adsorption phase and evaluate the extraction efficiency of three example explosives (or compounds associated with explosives and propellants) using the new phase.

The purpose of changing the adsorption phase was to investigate new intermolecular interactions between the sol-gel and the incoming explosive sample, similar to changing phases in column chromatography to increase or decrease interaction with the solute. The new sol-gel polymer containing 3-cyanopropyltrimethoxysilane (CMV-F) was created and is reported here for the first time. A detailed comparison of the extraction efficiencies between CMV-A and CMV-F for 1,3-Dinitrobenzene (1,3-DNB), 2,4-Dinitrotoluene (2,4-DNT), and Diphenylamine (DPA) through closed headspace sampling are reported. The listed explosives were chosen as they are commonly detected in Gunshot Residue (GSR) analysis, an application previously reported on using CMV-A. Future work will include a more in-depth look into the interactions between the cyanopropyl functional group and the three target compounds selected for this study. A Keyence® digital microscope was used to image the morphology of each phase and Fourier Transform Infrared (FTIR) was used to chemically characterize the sol-gel coating on the CMV-F glass filters. The results showed that CMV-F increased the recovery of DPA by one order of magnitude for a 75ng spike over CMV-A. These results are potentially useful for detection of extremely low (sub-ng) quantities for GSR since DPA is the most common organic compound residue found on the hands of persons who had recently fired a gun. The overall conclusions of this effort suggest that CMV-F has potential as an improvement for the extraction of other explosives and compounds associated with explosives and propellants.

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**Headspace Sampling, Explosives, Sol-Gel**



## Young Forensic Scientists Forum— 2019

### Y18 A New Assay for 1,3-Dimethylamylamine: An Emerging Drug of Abuse

Sandra M. Pieczonka\*, Loyola University Chicago, Crystal Lake, IL 60014

**Learning Overview:** After attending this presentation, attendees will be able to understand the dangers of 1,3 Dimethylamylamine (DMAA), learn about techniques that other scientists have used previous to this research, and discover how this research has changed the way this compound can be analyzed using easier methods and more inexpensive materials.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a new way of analyzing banned substances more accurately, more quickly, and more cost effectively.

The subject compound, DMAA, is marketed as a “natural” stimulant in nutritional supplements and is often found in weight loss and athletic performance-enhancing products. Recent studies have linked this amphetamine-like compound to cardiovascular problems such as shortness of breath, tightening in the chest, and possible heart attack. Consumption of DMAA, also known as methylhexanamine, geranamine, and 2-amino-4-methylhexane, has been linked to multiple deaths.<sup>1</sup> This compound was banned in 2010 by competitive sports authorities, such as the International Association of Athletics and the International Olympic Committee, both of which abide by the policies set forth by the World Anti-Doping Agency.<sup>2</sup> Any quantity of DMAA found in an athlete’s urine results in automatic suspension and sanction. In the past few years, DMAA has been identified in seized drug exhibits by the Drug Enforcement Administration (DEA).<sup>3</sup>

Qualitative and quantitative methods previously reported in the literature have used High-Performance Liquid Chromatography/Ultraviolet (HPLC/UV), Proton Nuclear Magnetic Resonance (<sup>1</sup>HNMR), Mass Spectrometry (MS), and Gas Chromatography (GC).<sup>4-7</sup> This new method uses GC/MS and Flame Ionization Detection (FID) to assay DMAA in commercial products, usually in the form of the hydrochloride salt (DMAA.HCl). Sample preparation consists of extracting DMAA.HCl with aqueous HCl in the range of 0.1M–0.5M followed by conversion of DMAA.HCl to the free base with aqueous sodium hydroxide (NaOH) solution and extraction into chloroform. On-column derivatization to the DMAA-alkanoyl amide was achieved in chloroform with the addition of a lower alkanoyl anhydride and triethylamine. Compared to the free base, DMAA-acyl amide derivatives have increased volatility and decreased surface activity, which results in enhanced chromatography, detector response, and specificity. Additionally, this new method does not require heating or sonication prior to analysis and uses readily available laboratory chemicals for derivatization, such as acetic, propanoic, and butanoic anhydrides. The use of various alkanoyl anhydrides provides method versatility across a broad product line and avoids interference from components in certain commercial products. Acylation of DMAA with a homologous series of anhydrides creates amides with predictably later retention times. Lastly, the resolution of the diastereomeric analyte pairs was enhanced as the molecular weight of the derivatizing agent increased.

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#### 1,3-dimethylamylamine, DMAA, Assay



## Young Forensic Scientists Forum— 2019

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### Y19 The Prevalence of Male DNA Under a Female's Fingernails

*Alexis Baxter, BS\*, Cedar Crest College, Allentown, PA 18104; Janine Kishbaugh, MS, Cedar Crest College, Allentown, PA 18104*

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**Learning Overview:** After attending this presentation, attendees will understand that male DNA under a female's fingernails holds probative value in casework when a full Y-chromosomal Short Tandem Repeat (Y-STR) profile is obtained. When a partial Y-STR profile is obtained, it is typically due to deposit during everyday activities.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing how, through the results obtained in this research, fingernail evidence collection and DNA analysis of this evidence will be affected and led into a direction of optimization.

The frequency of finding a male DNA profile under a female's fingernails is currently unknown by the scientific community. When a victim comes into contact with a male assailant during a violent or sexual assault, DNA analysis is performed to find the presence of foreign male DNA on the victim. Evidence is collected with a sexual assault evidence kit, which includes cutting, scraping, or swabbing of the victim's fingernails. However, finding male DNA may not necessarily reflect the context of how the transfer occurred and may not be the profile of the assailant. Considering that the sensitivity of DNA methodologies has improved, DNA analysis may be detecting previously undetected DNA that is actually being transferred through casual contact. In addition, secondary and even tertiary transfer may be detected. Previous studies have shown that the origin of foreign DNA under fingernails can be associated to cohabitants. Assessing the random nature of male-to-female transfer throughout the day is the basis for this study.

Y-STRs were used for typing of detected foreign DNA. Female participants in this study either lived with or frequently encountered male individuals in their daily lives. All samples were collected via cutting with fingernail clippers. All nails from one hand were combined and analyzed together. The fingernails were stored in a microcentrifuge tube and kept at -20°C until the samples were extracted. Extraction was performed on the fingernails using a QIAamp® DNA Investigator® kit combined with the Lyse&Spin kit. Quantitation was not performed in this study due to the amount of male DNA being extremely low. Amplification was performed with the PowerPlex® Y23 system. Genotyping was performed using an Applied BioSystems® 3130XL and fragment analysis was performed with GeneMapper® IDX v1.5. The sensitivity was set to 50 Relative Fluorescence Units (RFUs), and anything detected below that was not included in interpretation. Fifteen females participated in this study, resulting in 90 samples that were analyzed. Of the samples tested, partial profiles were produced from single and multiple contributors. In some cases, profiles were linked to male cohabitants via reference profiles provided. When the male cohabitant profile was detected, most allele calls were linked back to that individual. Since only partial profiles were obtained in this study, it is hypothesized that full male profiles obtained during casework may be more indicative of close, immediate contact between the victim and their assailant. Also, since the male cohabitant profile contributed to most allele calls, any foreign alleles present should be carefully interpreted.

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#### Fingernail Clippings, Y-STR, DNA



## Young Forensic Scientists Forum— 2019

### Y20 The Detection of Male DNA Using Y-Chromosomal Short Tandem Repeats (Y-STRs) in Post-Coital Samples of Vasectomized Males

Sarah Lighthart, BS\*, Cedar Crest College, Allentown, PA 18104; Janine Kishbaugh, MS, Cedar Crest College, Allentown, PA 18104

**Learning Overview:** After attending this presentation, attendees will be more knowledgeable about the potential Y-STR DNA results from extended collection intervals.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by becoming assisting attendees in becoming more knowledgeable and aware of the results of collecting azoospermic sexual assault samples at extended intervals.

In the United States, one in six women will be raped. The perpetrators of these crimes are male 94% of the time.<sup>1</sup> In the case of a male perpetrator and female victim, there is the potential for deposition of semen in the vaginal cavity; however, not all cases with semen present will contain sperm. A study reported that approximately one-third of men that commit sexual crimes suffer from some form of sexual dysfunction, which can result in a decrease or complete lack of sperm.<sup>2</sup> Ejaculate with a reduced amount of sperm vastly decreases the amount of DNA present for subsequent testing. This study sought to establish how long after an assault a viable DNA profile may be obtained from samples without sperm.

Semen without sperm was simulated using vasectomized male participants. A total of five vasectomized couples participated in the study by completing a collection packet. Each couple participated in four different trials using post-coital intervals of one, three, five, and seven days. In each trial, the female swabbed the vaginal cavity twice. During each insertion, two swabs were used simultaneously for a total of four swabs for each interval. The two swabs inserted together were extracted together using a solid phase extraction kit. Low Copy Number (LCN) procedures were utilized to help detect any low-level amount of DNA in the sample. Quantitation was performed using a commercial kit that detects the total DNA concentration as well as male DNA. No quantitation results were obtained; however, the samples were amplified with a commercial Y-STR kit. The resulting DNA profiles were analyzed to determine the total number of male-specific alleles present for each time interval. After one day, the results from five couples contained an average of 20.6 of 23 alleles (89.5%), ranging from 13 to 23 alleles present. The average number of alleles found after three days was 4.4 (19.1%) with a range of 0 to 10 alleles present. An average of 3.2 (13.9%) alleles were present after five days with a range of 0 to 8 alleles. After seven days, the average number of alleles identified was 0.8 (3.4%) with a range of 0 to 2 alleles present. Thus, the time frame to detect nearly full DNA profiles from samples without the presence of sperm is approximately one day post-coitus based on the current results. Though full profiles may not be obtained, the presence of alleles can aid in suspect elimination. Future work utilizing LCN methods may increase the amount of alleles obtained from longer intervals.

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#### Sexual Assault, DNA, Y-Chromosome Short Tandem Repeat



## Young Forensic Scientists Forum— 2019

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### Y21 The Implementation of a Method for Rapid Direct Polymerase Chain Reaction (PCR) on Diluted Saliva

Mallory Baud, BS\*, Miami, FL 33174; Georgiana C. Gibson-Daw, PhD, Lincoln, NE 68521; Bruce R. McCord, PhD, Florida International University, Miami, FL 33199

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**Learning Overview:** After attending this presentation, attendees will better understand a method for rapid and direct PCR on diluted direct saliva.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing results that show how run time and reagent costs can be reduced using this combined rapid and direct PCR method. This project will improve current forensic DNA analysis by reducing time and steps involved in sample analysis. The removing of the extraction step in sample analysis decreases extraction-based sample loss as well as potential lab contamination due to sample transfer steps. Attendees will see results of an experimental design-based method to optimize direct and rapid PCR amplification of diluted saliva. These results provide a procedure to reduce the time of the PCR leading to the rapid production of a 7-locus DNA profile for sample screening. This method can be very useful for suspect screening, (e.g., booking stations, customs, and mass disasters).

While current DNA analysis procedures are highly automated, multiplexed, and efficient, they are relatively slow on a per-sample basis. Thus, there is still a need for rapid screening of single-suspect samples. One solution to reduce analysis time is to use rapid direct PCR, which combines cell extraction and PCR amplification in the same tube. Rapid direct PCR procedures can greatly reduce the processing time by screening suspects for presumptive results. This decrease in processing time and reagent use leads to a quick turnaround and inexpensive processing of large numbers of samples.

In order to do this, specially engineered enzymes, such as OmniTaq (a triple mutant of Taq polymerase that makes it resistant to inhibitory effects of body fluids and soils), and high-speed thermal cyclers, capable of temperature changes as fast as 15 degrees per second, will be used to process a specifically designed 7-locus multiplex. The goal is to reduce the analysis time to less than ten minutes by elimination of the extraction step. The MP7 primers consist of the following Short Tandem Repeat (STR) markers: D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, and amelogenin loci. These loci cover from 100 to 450 base pair. Work was also performed on a miniSTR multiplex consisting of TH01, FGA, CSF1PO, D21S11, D5S818, D7S820, TPOX, D18S1, and amelogenin—STR loci that cover 51 to 281 bases. These shorter loci are suspected of needing less time to amplify. Those primers, being much smaller, would reduce the annealing time leading to a shorter reaction. Next, 2 $\mu$ L of DNA is added to 8 $\mu$ L of PCR reaction, which is amplified during 32 cycles at 98°C for 3 seconds and 62°C for 14 seconds. Once amplified, the sample is run on ABI® Prism® 310 Genetic Analyzer or a microfluidic device. The PCR reaction mixture was optimized using experimental mixture designs. The experimental design considered salt concentration, buffer addition, dNTP addition, primer addition, and PCR enhancers to develop experiments to maximize amplification of straight saliva. Those modifications created a faster PCR reaction which was obtained in 12 minutes 36 seconds. The output was optimized base on the overall peak heights of a 7-locus multiplex.

The result of this study demonstrates the application of rapid direct PCR for the analysis of STRs to obtain a presumptive DNA profile in under 13 minutes. Without any extraction, the PCR reagents and enhancers have been shown to work with the inhibitors present in saliva.

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#### Rapid PCR, Direct PCR, Saliva



## Young Forensic Scientists Forum— 2019

### Y22 The Effect of Demographic Factors and Mortality-Related Health Conditions on Postmortem Biometric Data

*Samantha Upton, BA\*, University of Tennessee, Knoxville, TN 36996; Anielle Duncan, BA\*, University of Tennessee, Knoxville, TN 37996; Elizabeth Albee, BA, University of Tennessee, Knoxville, TN 37996; Kelly Sauerwein, PhD, Standards Coordinating Body, Gaithersburg, MD 20899; Dawnie W. Steadman, PhD, University of Tennessee, Dept of Anthropology, Knoxville, TN 37996*

**Learning Overview:** After attending this presentation, attendees will understand the influence of demographic and health-related variables on the deterioration of fingerprints, which are commonly used as a biometric indicator.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by clarifying the effect of sex, age, and various antemortem health conditions on the quality of postmortem biometric data that are commonly relied upon to obtain positive identifications for previously unknown individuals.

Biometrics are measurable unique physiological or behavioral characteristics that are used to classify both living and deceased individuals. Previous research has indicated that fingerprints are comparatively the most successful biometric indicator for positive identification, generally due to the longevity of postmortem fingerprint capture. Therefore, in this study, fingerprints were used as the primary indicator to examine biometric degradation and quality data acquisition. For the purposes of this study, *quality data* refers to a threshold met by a ratio of numerical scores provided by two types of biometric scanning technologies. Fingerprint quality was determined by the National Institute for Scientific Standards (NIST) Fingerprint Image Quality (FIQ) v.2.0 algorithm where prints are scored on a 1-5 scale, with 1-3 denoting adequate to excellent prints and 4-5 denoting poor quality prints. As the purpose of the scanning technologies is to provide a match (i.e., positive identification) based on biometric indicators, the scores predict the likelihood that the fingerprint image meets the quality threshold to achieve a positive match. Along with researcher experience, these numerical scores are considered when determining enrollment for longitudinal postmortem studies at the University of Tennessee's Anthropology Research Facility. Biometric scanning technologies were used to capture digital images of all ten digits, where applicable, of 150 donated individuals upon their initial intake into the William M. Bass Body Donation program between April 2014 and July 2018. If 50% or more of the digits were at or above adequate quality (i.e., NFIQ score between 1-3), the individual was then accepted for longitudinal biometric research. Though 150 individuals, between the ages 18 and 94, have been scanned from the beginning of this study, less than 50% met the quality threshold for longitudinal data collection. Therefore, this study examines the correlation between certain demographic variables (e.g., sex and age), mortality-inducing health conditions (e.g., cancers, diabetes, heart disease, pulmonary disease), and other significant conditions (e.g., smoking and alcoholism) with the quality of biometric data during the postmortem period. For each of the 150 individuals in this study, data were collected from the Forensic Anthropology Center's donation database on the following variables: sex, age-at-death, Cause Of Death (COD), and self-reported medical history. The goal of this study was to examine the impact of these variables to overall fingerprint quality.

The preliminary results of this study show that while biometrics do remain viable over time, age was the most significant factor affecting the retention of identifying features of the fingerprints. Among individuals aged 70 years and older ( $n=75$ ), 66% ( $n=50$ ) were rejected for longitudinal enrollment. Similarly, 64% ( $n=7$ ) of individuals 60 years and less ( $n=11$ ) scanned met or exceeded the quality threshold and, therefore, were eligible for further data collection. However, proportions of individuals between 60 and 70 years of age are relatively equally distributed between enrolled and not enrolled categories. Explanations for such results warrant further analysis. There were no substantial differences when sex of individuals and fingerprint quality were examined (enrolled/not enrolled females:  $n=33/32$ ; enrolled/not enrolled males:  $n=39/46$ ). Furthermore, this study has yet to show any apparent trends in the relationship between health-related conditions and fingerprint quality; however, analyses are ongoing. This study builds upon previous work and continues to support the utility of physiological biometric identifiers to obtain positive identifications in postmortem contexts. Postmortem biometric research has the potential to make important contributions to forensic anthropology and the law enforcement, military, and medicolegal communities.

#### Biometrics, Demographics, Mortality



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Jonathan D. Bethard, PhD – A28, A35, A113, A137

Discloses no financial relationships with commercial entities.

Marianne E. Beynon, MD – H119

Discloses no financial relationships with commercial entities.

Zumrad Usman Bhutta, MS – E86

Discloses no financial relationships with commercial entities.

Mubarak A. Bidmos, PhD – A10

Discloses no financial relationships with commercial entities.

Frederick R. Bieber, PhD – F4

Discloses no financial relationships with commercial entities.

Hannah Elyse Bielamowicz, MD – H187

Discloses no financial relationships with commercial entities.

Stanislaw T.J. Bielous, BS – E94

Discloses no financial relationships with commercial entities.

Julie Binette, BS – J13

Discloses no financial relationships with commercial entities.

Sandra C. Bishop-Freeman, PhD – K69

Discloses no financial relationships with commercial entities.

Chawki Bisker, MSc – E121, H99

Discloses no financial relationships with commercial entities.

Alissa L. Bjerkhoel, JD – F17

Discloses no financial relationships with commercial entities.

Scott Blair, JD – W17

Discloses no financial relationships with commercial entities.

Brooke H. Blake, MD – H190

Discloses no financial relationships with commercial entities.

Katie E. Blamar, BS – B31

Discloses no financial relationships with commercial entities.

Melissa M. Blessing, DO – H118

Discloses no financial relationships with commercial entities.

Jatin Bodwal, MD – D34

Discloses no financial relationships with commercial entities.

Jesper L. Boldsen, PhD – W24

Discloses no financial relationships with commercial entities.

Katelyn L. Bolhofner, PhD – A135

Discloses no financial relationships with commercial entities.

Timothy Bollé – C26

Discloses no financial relationships with commercial entities.

Omar Bonato, MD – E20

Discloses no financial relationships with commercial entities.

Julian C.W. Boon, PhD – I20

Discloses no financial relationships with commercial entities.

Jason A. Bordelon – W05

Discloses no financial relationships with commercial entities.

Trevor A. Borja, BA – B117

Discloses no financial relationships with commercial entities.

Matteo Borrini, PhD – LW3

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Lisa Borsuk, MS – B2

Discloses no financial relationships with commercial entities.

David R. Bosch, PhD – D22, D27

Discloses no financial relationships with commercial entities.

Caterina Bosco, MD – E11

Discloses no financial relationships with commercial entities.

Sabra R. Botch-Jones, MS – K13

Discloses no financial relationships with commercial entities.

Fatimah Bouderdaben – A149

Discloses no financial relationships with commercial entities.

Jennifer W. Bourgeois, MS – F39

Discloses no financial relationships with commercial entities.

Derek A. Boyd, MA – A63

Discloses no financial relationships with commercial entities.

Donna C. Boyd, PhD – A138

Discloses no financial relationships with commercial entities.

Cody L. Braley – B33

Discloses no financial relationships with commercial entities.

Helen M. Brandt, MS – A8

Discloses no financial relationships with commercial entities.

Sophia Brathwaite, BSc – K71

Discloses no financial relationships with commercial entities.

Jennifer Gombos Breaux, MFS – W10

Discloses no financial relationships with commercial entities.

Charles H. Brenner, PhD – B76

Discloses no financial relationships with commercial entities.

Amber N. Bridges, MD – I2

Discloses no financial relationships with commercial entities.

Kendra Broadwater, MS – W23

Discloses no financial relationships with commercial entities.

Connor Brooks, MSc – K81

Discloses no financial relationships with commercial entities.

Helmut G. Brosz, BASc – D28, D29, D32

Discloses no financial relationships with commercial entities.

Catherine O. Brown, MSFS – B6

Discloses no financial relationships with commercial entities.

Elizabeth A. Brown – W15

Discloses no financial relationships with commercial entities.

Katherine M. Brown, PhD – E32, W15

Discloses no financial relationships with commercial entities.

Melissa Ann Brown, MA – A27

Discloses no financial relationships with commercial entities.

Tracy E. Brown – A66

Discloses no financial relationships with commercial entities.

Joshua L. Brunty, MS – C20

Discloses no financial relationships with commercial entities.

Lori Bruski, BS – W19

Discloses no financial relationships with commercial entities.

Cynthia Brzozowski, DMD – G4

Discloses no financial relationships with commercial entities.

Sydney R. Buchalter, BS – B179

Discloses no financial relationships with commercial entities.

Kristi Bugajski, PhD – H89

Discloses no financial relationships with commercial entities.

Brandon J. Bukovitz, BS – H65

Discloses no financial relationships with commercial entities.

Ted M. Burkes, BS – J34

Discloses no financial relationships with commercial entities.

Amber Burns, MS – W23

Discloses no financial relationships with commercial entities.

JoAnn Buscaglia, PhD – B95, B159

Discloses no financial relationships with commercial entities.

Daniel C. Butler, MD – H169

Discloses no financial relationships with commercial entities.

John M. Butler, PhD – W10

Discloses no financial relationships with commercial entities.

Nasir Butt, PhD – B184

Discloses no financial relationships with commercial entities.

Patrick Buzzini, PhD – B166, J10

Discloses no financial relationships with commercial entities.

Krystal T. Byrd, BSc – B138

Discloses no financial relationships with commercial entities.

Jennifer F. Byrnes, PhD – A131

Discloses no financial relationships with commercial entities.

Joan A. Bytheway, PhD – A62

Discloses no financial relationships with commercial entities.

### C

Mary E. Cablk, PhD – F18

Discloses no financial relationships with commercial entities.

Joaquin Cabrera, JD – W23

Discloses no financial relationships with commercial entities.

Crystal Cafiero, MFS – F11

Discloses no financial relationships with commercial entities.

Matthew D. Cain, MD – H41

Discloses no financial relationships with commercial entities.

Enrica Calabrese, MD – H84

Discloses no financial relationships with commercial entities.

Cynthia Cale, MS – F8

Discloses no financial relationships with commercial entities.

Melina Calmon Silva, MA – A130

Discloses no financial relationships with commercial entities.

Annabelle C. Campbell, BSc – B81

Discloses no financial relationships with commercial entities.

Justine Canales, MD – H127

Discloses no financial relationships with commercial entities.

Meta Y. Candra, MSc – H1

Discloses no financial relationships with commercial entities.

Kris C. Cano, MA – W12

Discloses no financial relationships with commercial entities.

Erica Cantor, BS – B124

Discloses no financial relationships with commercial entities.

Michael J. Caplan, MD – ES1

Discloses no financial relationships with commercial entities.

Fiorella Caputo, MD – H63

Discloses no financial relationships with commercial entities.

Felice F. Carabellese, MD – I1

Discloses no financial relationships with commercial entities.

Matilde J. Carbia, JD – F40

Discloses no financial relationships with commercial entities.

Eduardo M. Cardoso, MSc – K27

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Shelby Carlson – B10

Discloses no financial relationships with commercial entities.

Douglas J. Carpenter, MS – D25

Discloses no financial relationships with commercial entities.

Morgan N. Carpenter, BS – B136

Discloses no financial relationships with commercial entities.

Alicia L. Carriquiry, PhD – B160

Discloses no financial relationships with commercial entities.

Marla E. Carroll, BS – C10, C21

Discloses no financial relationships with commercial entities.

Henry J. Carson, MD – H180

Discloses no financial relationships with commercial entities.

Claire M. Cartozzo, MS – H30

Discloses no financial relationships with commercial entities.

James Louis Caruso, MD – W04

Discloses no financial relationships with commercial entities.

Mary E.S. Case, MD – W17

Discloses no financial relationships with commercial entities.

Eoghan Casey, PhD – C4, C5, C22

Discloses no financial relationships with commercial entities.

Jason A. Casper, MFS – B66

Discloses no financial relationships with commercial entities.

Rudy J. Castellani, MD – H124, S02

Discloses no financial relationships with commercial entities.

Joseph Cavise, JD – F28

Discloses no financial relationships with commercial entities.

Oktay Cavus, MSc – F3, I15

Discloses no financial relationships with commercial entities.

Greta Cena, MD – E14

Discloses no financial relationships with commercial entities.

Jessica I. Cerezo-Román, PhD – A129

Discloses no financial relationships with commercial entities.

Scott Chadwick, PhD – B183

Discloses no financial relationships with commercial entities.

Sarah E. Chaffman – B65

Discloses no financial relationships with commercial entities.

Reba E. Chamblee – K37

Discloses no financial relationships with commercial entities.

Carole E. Chaski, PhD – D8, F14, J18

Discloses no financial relationships with commercial entities.

Vasiliki Chatzaraki, MD – H109, H154

Discloses no financial relationships with commercial entities.

Muhammad Taimoor Chaudhary, MPhil – K3

Discloses no financial relationships with commercial entities.

Jennett M. Chenevert, MS – K58

Discloses no financial relationships with commercial entities.

Chao-Chun C. Cheng – C19

Discloses no financial relationships with commercial entities.

Abigail Chesness – H130

Discloses no financial relationships with commercial entities.

Lesley A. Chesson, MS – A74

Discloses no financial relationships with commercial entities.

Kemp Chester, MA – K81

Discloses no financial relationships with commercial entities.

Irene M. Chiang, BS – B149

Discloses no financial relationships with commercial entities.

Anchalee Chittamma – H138

Discloses no financial relationships with commercial entities.

Young Jin Cho – D41

Discloses no financial relationships with commercial entities.

Kwangsoo Choi, MA – D9

Discloses no financial relationships with commercial entities.

Alexander F. Christensen, PhD – A58

Discloses no financial relationships with commercial entities.

Angi M. Christensen, PhD – A54

Discloses no financial relationships with commercial entities.

Sheresa Christopher, PhD – I33

Discloses no financial relationships with commercial entities.

Sarah Chu, MS – F15

Discloses no financial relationships with commercial entities.

Hee-Sun Chung, PhD – K5, K6, K7

Discloses no financial relationships with commercial entities.

Jae W. Chung, BS – W5

Discloses no financial relationships with commercial entities.

Elizabeth Church – A134

Discloses no financial relationships with commercial entities.

Dennis J. Chute, MD – H177

Discloses no financial relationships with commercial entities.

Maria Susana Ciruzzi, PhD – F42

Discloses no financial relationships with commercial entities.

Michelle S. Clark, MS – H132

Discloses no financial relationships with commercial entities.

Randall Clark, PhD – B177

Discloses no financial relationships with commercial entities.

Chaunesey Clemmons, BA – A148

Discloses no financial relationships with commercial entities.

David W. Coates, MS – H147

Discloses no financial relationships with commercial entities.

Michael D. Coble, PhD – F6

Discloses no financial relationships with commercial entities.

Jill H. Cocking, MSc – B198

Discloses no financial relationships with commercial entities.

Stephen D. Cohle, MD – W07

Discloses no financial relationships with commercial entities.

Simon Cole, PhD – B98

Discloses no financial relationships with commercial entities.

Alexander J. Colla, MFS – B70

Discloses no financial relationships with commercial entities.

Kerry A. Collins, JD – F10

Discloses no financial relationships with commercial entities.

Caroline Concannon, BSN – E13

Discloses no financial relationships with commercial entities.

Marta Concheiro-Guisan, PhD – K56

Discloses no financial relationships with commercial entities.

Aime Conigliaro, MSc – G32

Discloses no financial relationships with commercial entities.

Jillian Conte, PhD – E125

Discloses no financial relationships with commercial entities.

Mauro Coppone, MD – E20

Discloses no financial relationships with commercial entities.

Fabrizio Cordasco, MD – H151, I39

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Tracey S. Corey, MD – H195

Discloses no financial relationships with commercial entities.

Jered B. Cornelison, PhD – H146

Discloses no financial relationships with commercial entities.

Helena D. Corro, MD – H53

Discloses no financial relationships with commercial entities.

Louise K. Corron, PhD – A108

Discloses no financial relationships with commercial entities.

Ruthmara Corzo – E101

Discloses no financial relationships with commercial entities.

Jenny Cossham, MS – W15

Discloses no financial relationships with commercial entities.

Marcus de Abreu Farias Costa – B134

Discloses no financial relationships with commercial entities.

Robin W. Cotton, PhD – W10

Discloses no financial relationships with commercial entities.

Curtis B. Coulter, JD – W17

Discloses no financial relationships with commercial entities.

Joseph A. Cox, MS – K36

Discloses no financial relationships with commercial entities.

Amy M. Crawford, MS – J29

Discloses no financial relationships with commercial entities.

Mark W. Crumpton, DMD – G13

Discloses no financial relationships with commercial entities.

Luis A. Cuadra-Rodriguez, BS – B54

Discloses no financial relationships with commercial entities.

Breanna M. Cuchara, MFS – E63, H76

Discloses no financial relationships with commercial entities.

Eugenia Cunha, PhD – A94

Discloses no financial relationships with commercial entities.

Serena Maria Curti, MD – E11

Discloses no financial relationships with commercial entities.

Donatella Curtotti – F41

Discloses no financial relationships with commercial entities.

### D

Corinne D'Anjou, DMD – G12

Discloses no financial relationships with commercial entities.

Ronaldo C. da Silva, Jr., PhD – B192

Discloses no financial relationships with commercial entities.

Veronica B. Dahir, PhD – J2, J6

Discloses no financial relationships with commercial entities.

Bin B. Dai – H135

Discloses no financial relationships with commercial entities.

Kadri Dalgic, MAFS – I15

Discloses no financial relationships with commercial entities.

James Darnell, BS – W06

Discloses no financial relationships with commercial entities.

J. Tyler Davidson, MS – B171

Discloses no financial relationships with commercial entities.

Marion E. Davidson – A44

Discloses no financial relationships with commercial entities.

Kymeri E. Davis, BSc – K10

Discloses no financial relationships with commercial entities.

Lucy A. Davis, BHS – W12

Discloses no financial relationships with commercial entities.

Reed A. Davis, MSc – A55

Discloses no financial relationships with commercial entities.

Josep De Alcaraz-Fossoul, PhD – B69

Discloses no financial relationships with commercial entities.

Luigi De Aloe, MD – H152, I39

Discloses no financial relationships with commercial entities.

Hans H. De Boer, MD, PhD – A82, A83

Discloses no financial relationships with commercial entities.

Eduardo G. de Campos, MSc – K35

Discloses no financial relationships with commercial entities.

Dean Michael De Crisce, MD – I22, S02

Discloses no financial relationships with commercial entities.

Marco De Donno – B96

Discloses no financial relationships with commercial entities.

Peter R. De Forest, DCrim – B199, B200

Discloses no financial relationships with commercial entities.

Gianni De Giorgio – E16

Discloses no financial relationships with commercial entities.

Stefania De Simone, MD – H10

Discloses no financial relationships with commercial entities.

Jannick De Tobel, MD – A111, G18

Discloses no financial relationships with commercial entities.

Alyssa Dean, MS – H174

Discloses no financial relationships with commercial entities.

Theresa DeAngelo, MS – W23

Discloses no financial relationships with commercial entities.

Joshua S. DeBord, PhD – B175

Discloses no financial relationships with commercial entities.

Summer J. Decker, PhD – H114

Discloses no financial relationships with commercial entities.

Heather L. Deel – H94

Discloses no financial relationships with commercial entities.

Beatrice Defraia – H21

Discloses no financial relationships with commercial entities.

Heather H. Degan, PhD – F43

Discloses no financial relationships with commercial entities.

Laurny DeGreeff, PhD – E29, E30

Discloses no financial relationships with commercial entities.

Joyce L. deJong, DO – H195

Discloses no financial relationships with commercial entities.

Alexia Delbreil – I11

Discloses no financial relationships with commercial entities.

Massimiliano dell'Aquila, MD – H80

Discloses no financial relationships with commercial entities.

John P. Demas, DDS – G3

Discloses no financial relationships with commercial entities.

Vaishali S. Deo, MD – H167

Discloses no financial relationships with commercial entities.

Randi Marie Depp, BS – A19

Discloses no financial relationships with commercial entities.

Victoria R. DePrimo, BS – B16

Discloses no financial relationships with commercial entities.

Shannon L. Dery – A51

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Lynette Desjarlais, BA – H39

Discloses no financial relationships with commercial entities.

Vincent J. Desiderio, Jr., - W12

Discloses no financial relationships with commercial entities.

Josh Dettman – B152

Discloses no financial relationships with commercial entities.

Khody R. Detwiler, BS – J16

Discloses no financial relationships with commercial entities.

Delilah DeWilde, BS – B174

Discloses no financial relationships with commercial entities.

Aadhar R. Dhamecha, MD – I30

Discloses no financial relationships with commercial entities.

Aldo Di Nunzio – K22

Discloses no financial relationships with commercial entities.

Ciro Di Nunzio, PhD – K22

Discloses no financial relationships with commercial entities.

Michele Di Nunzio, BS – K22

Discloses no financial relationships with commercial entities.

Mariantonia Di Sanzo, MD – F32

Discloses no financial relationships with commercial entities.

Giancarlo Di Vella, MD, PhD – E3, E5, E11, E12, E14

Discloses no financial relationships with commercial entities.

Peter J. Diaczuk – B68, F47

Discloses no financial relationships with commercial entities.

Francis X. Diamond, BS – W05

Discloses no financial relationships with commercial entities.

Francisco J. Diaz, MD – E123

Discloses no financial relationships with commercial entities.

Gregory M. Dickinson, MD – H69

Discloses no financial relationships with commercial entities.

Elizabeth A. DiGangi, PhD – A20, A5, A45, A46

Discloses no financial relationships with commercial entities.

Dennis C. Dirkmaat, PhD – A65

Discloses no financial relationships with commercial entities.

Stephanie Diu, BA – H171

Discloses no financial relationships with commercial entities.

Lawrence A. Dobrin, DMD – W19

Discloses no financial relationships with commercial entities.

Julia A. Dolan, MS – W18

Discloses no financial relationships with commercial entities.

Michael J. Dolan, Jr., MS – B165

Discloses no financial relationships with commercial entities.

Matthew Domanic, BA – W06

Discloses no financial relationships with commercial entities.

David Domingues Pavanelli, PhD – D35

Discloses no financial relationships with commercial entities.

Stephanie Domitrovich, JD, PhD – F24, F44, J21, S01, W17

Discloses no financial relationships with commercial entities.

John Donahue, MA – B185

Discloses no financial relationships with commercial entities.

Francesca Donno, MD – F13, H19, H20

Discloses no financial relationships with commercial entities.

Meryle A. Dotson, MA – E19

Discloses no financial relationships with commercial entities.

Sarah Dowling, BS – B197

Discloses no financial relationships with commercial entities.

James J.C.U. Downs, MD – BS6, W08, W11

Discloses no financial relationships with commercial entities.

Jay Doyle, PhD – C8

Discloses no financial relationships with commercial entities.

Jasmine M. Drake, PhD – F39

Discloses no financial relationships with commercial entities.

Mark S. Dreyfuss, PhD – B101

Discloses no financial relationships with commercial entities.

Gwenola Drogou, DDS – G32

Discloses no financial relationships with commercial entities.

Lena M. Dubois, MSc – B32, H92

Discloses no financial relationships with commercial entities.

Beatrix Dudzik, PhD – A127

Discloses no financial relationships with commercial entities.

Anielle Duncan, BA – Y22

Discloses no financial relationships with commercial entities.

Rhian Dunn, MS – A5

Discloses no financial relationships with commercial entities.

Tim G. Dunn, MS – D33

Discloses no financial relationships with commercial entities.

Tyler E. Dunn, MS – A2

Discloses no financial relationships with commercial entities.

### E

Katherine E. Eames – B71

Discloses no financial relationships with commercial entities.

Lars C. Ebert, PhD – H114

Discloses no financial relationships with commercial entities.

Heather J.H. Edgar, PhD – A104

Discloses no financial relationships with commercial entities.

Suni M. Edson, MS – B38, B40

Discloses no financial relationships with commercial entities.

Christopher J. Ehrhardt, PhD – B164

Discloses no financial relationships with commercial entities.

Heidi Eldridge, MS – B201

Discloses no financial relationships with commercial entities.

Pedro M.S. Eleuterio, MSc – C13, C16

Discloses no financial relationships with commercial entities.

Kelly M. Elkins, PhD – B8

Discloses no financial relationships with commercial entities.

Alexandra L. Emmons, MA – A53

Discloses no financial relationships with commercial entities.

Sandra R. Enslow, BA – E37

Discloses no financial relationships with commercial entities.

Brandon Epstein, BS – C21

Discloses no financial relationships with commercial entities.

Marianna Eserman, MD – W20

Discloses no financial relationships with commercial entities.

Ashley Espinoza – A12

Discloses no financial relationships with commercial entities.

Massimiliano Esposito, MD – F33

Discloses no financial relationships with commercial entities.

Elizabeth A. Evangelou, MA – A20, A45, A46

Discloses no financial relationships with commercial entities.





## Presenting Author Financial Disclosure – 2019

Kierstyn L. Evans, BS – B145

Discloses no financial relationships with commercial entities.

Brandon V. Everhart, BS – E49

Discloses no financial relationships with commercial entities.

Shari G. Ex, BA – A128

Discloses no financial relationships with commercial entities.

### F

Glenn P. Fahrig – B9

Discloses no financial relationships with commercial entities.

Anthony B. Falsetti, PhD – A48

SEM-EDX, Tescan, Vega 3 (Discussion of Unlabeled/  
Investigational Use of Product/Device).

Armin A. Farid, DMD – G8

Discloses no financial relationships with commercial entities.

Amanda L. Farrell, PhD – W01

Discloses no financial relationships with commercial entities.

Diana K. Faugno, MSN – B37

Discloses no financial relationships with commercial entities.

Matteo Favia – K28

Discloses no financial relationships with commercial entities.

Gerald Feigin, MD – H142

Discloses no financial relationships with commercial entities.

Khalid S. Feras, MPhil – E86

Discloses no financial relationships with commercial entities.

Joseph Ferencz, MD – I32

Discloses no financial relationships with commercial entities.

Morgan J. Ferrell – A113

Discloses no financial relationships with commercial entities.

Alessandro M. Ferrero, MSc – D21

Discloses no financial relationships with commercial entities.

Federica Fersini – I34

Discloses no financial relationships with commercial entities.

Martina Fichera, MD – F33

Discloses no financial relationships with commercial entities.

Marisia A. Fikiet, MS – E81

Discloses no financial relationships with commercial entities.

Costanza Filomena – E10, E28

Discloses no financial relationships with commercial entities.

Oran Finegan, MSc – A31

Discloses no financial relationships with commercial entities.

Marissa J. Finkelstein, MS – K72

Discloses no financial relationships with commercial entities.

Janet E. Finlayson, MA – A115

Discloses no financial relationships with commercial entities.

Tafs R. Fiorentin, PhD – B27

Discloses no financial relationships with commercial entities.

Amanda O. Fisher-Hubbard, MD – H193

Discloses no financial relationships with commercial entities.

Colleen M. Fitzpatrick, PhD – LW6

Discloses no financial relationships with commercial entities.

Julie M. Fleischman, PhD – A85, A142

Discloses no financial relationships with commercial entities.

Allie Flores, BS – B5

Discloses no financial relationships with commercial entities.

Kathleen Flor-Stagnato, MA – A67

Discloses no financial relationships with commercial entities.

Megan M. Foley, MSFS – B49

Discloses no financial relationships with commercial entities.

Thomas P. Forbes, PhD – B103, B178

Discloses no financial relationships with commercial entities.

Jessica R. Ford - Y15

Discloses no financial relationships with commercial entities.

Luisa Forger, MS – H96

Discloses no financial relationships with commercial entities.

Alexander Robert W. Forrest, LLM – K80

Discloses no financial relationships with commercial entities.

Allegra N. Forwith, BA – E45

Discloses no financial relationships with commercial entities.

Kristen L. Fowble, BS – E87

Discloses no financial relationships with commercial entities.

David R. Fowler, MD – K80

Discloses no financial relationships with commercial entities.

Kaitlyn Fox, BS – C7

Discloses no financial relationships with commercial entities.

Darren Franck, MSME – D20

Discloses no financial relationships with commercial entities.

Harold Franck, MSEE – D20

Discloses no financial relationships with commercial entities.

Kelvin J. Frank, Jr., BS – B140

Discloses no financial relationships with commercial entities.

Annarita Franza, PhD – F2, I3, LW2

Discloses no financial relationships with commercial entities.

Roger Frappier, MSc – W10

Discloses no financial relationships with commercial entities.

Eric Frauenhofer – B56

Discloses no financial relationships with commercial entities.

Jamie D. Fredericks, PhD – B3

Discloses no financial relationships with commercial entities.

Josh Friedman, MD, PhD – W03

Discloses no financial relationships with commercial entities.

Amanda N. Friend, MA – A116

Discloses no financial relationships with commercial entities.

Shuntaro Fujimoto, MS – B51

Discloses no financial relationships with commercial entities.

Christine Funk, JD – S02, W12

Discloses no financial relationships with commercial entities.

### G

Davette N. Gadison, MA – A24

Discloses no financial relationships with commercial entities.

Jasna Galekovic – J4, J33

Discloses no financial relationships with commercial entities.

Tim Gallagher, MD – W02

Discloses no financial relationships with commercial entities.

Shawna F. Gallegos – H26

Discloses no financial relationships with commercial entities.

Charles M. Gammill, BA – H48

Discloses no financial relationships with commercial entities.

Jan C. Garavaglia, MD – W08

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Sara N. Garcia – A56

Discloses no financial relationships with commercial entities.

Zoe S. Garcia, BSc – B127

Discloses no financial relationships with commercial entities.

Brett O. Gardner, PhD – E112

Discloses no financial relationships with commercial entities.

Taylor L. Gardner, BfSc – B196, E4, I13, G40

Discloses no financial relationships with commercial entities.

Nathaniel M. Garton, MSc – B142

Discloses no financial relationships with commercial entities.

Heather M. Garvin, PhD – A40

Discloses no financial relationships with commercial entities.

Shelby Garza, BS – A50

Discloses no financial relationships with commercial entities.

Sidney Gaston Sanchez – B106

Discloses no financial relationships with commercial entities.

Vittorio Gatto, MD – H79

Discloses no financial relationships with commercial entities.

Quentin T. Gauthier, MSFS – B122

Discloses no financial relationships with commercial entities.

Irina Geiman, MS – J14

Discloses no financial relationships with commercial entities.

Alexa Genalo, MSc – K53

Discloses no financial relationships with commercial entities.

Patrick H. Geoghegan, PhD – D3

Discloses no financial relationships with commercial entities.

Rebecca L. George, MA – W22

Discloses no financial relationships with commercial entities.

Charles E. Georget, PhD – G32

Discloses no financial relationships with commercial entities.

Zeno J. Geradts, PhD – C24, W17, W28

Discloses no financial relationships with commercial entities.

Krysalyn C. Gerhardt, BS – B73

Discloses no financial relationships with commercial entities.

Sara M. Getz, PhD – A25, W24

Discloses no financial relationships with commercial entities.

Keylie M. Gibson, BS – B109

Discloses no financial relationships with commercial entities.

Mark A. Giffen, Jr., DO – H74, H75

Discloses no financial relationships with commercial entities.

Ashley A. Gilbert, BS – K18

Discloses no financial relationships with commercial entities.

Michael D. Gilchrist, PhD, DEng – D15

Discloses no financial relationships with commercial entities.

James R. Gill, MD – H46, H195

Discloses no financial relationships with commercial entities.

Rachel B. Gilmore – B48

Discloses no financial relationships with commercial entities.

Cinzia Gimelli, PsyD, PhD – I13

Discloses no financial relationships with commercial entities.

Lorenzo Gitto, MD – H55, H73, H116, H123, H125, H139

Discloses no financial relationships with commercial entities.

Gabriella H. Giudice, BSc – K79

Discloses no financial relationships with commercial entities.

Oliver Giudice, PhD – C12, C23, C28, F41

Discloses no financial relationships with commercial entities.

Matthew C. Go, MA – A88

Discloses no financial relationships with commercial entities.

Timothy P. Gocha, PhD – A125

Discloses no financial relationships with commercial entities.

Zachary C. Goecker, MPS – B79

Discloses no financial relationships with commercial entities.

Mark Goff, BA – W09

Discloses no financial relationships with commercial entities.

Jae-mo Goh, PhD – D42

Discloses no financial relationships with commercial entities.

Vidia A. Gokool, BSc – B92

Discloses no financial relationships with commercial entities.

Bruce A. Goldberger, PhD – BS4, W08

Discloses no financial relationships with commercial entities.

Julietta Gomez Garcia-Donas – A29

Discloses no financial relationships with commercial entities.

John V. Goodpaster, PhD – B87

Discloses no financial relationships with commercial entities.

Jan M. Gorniak, DO – H48

Discloses no financial relationships with commercial entities.

Ludovica Gorza, MSc – G27

Discloses no financial relationships with commercial entities.

Emily D. Gottfried, PhD – I24

Discloses no financial relationships with commercial entities.

Lynsey F. Gozna, PhD – I31

Discloses no financial relationships with commercial entities.

Silke Grabherr, PhD – W07

Discloses no financial relationships with commercial entities.

Michael A. Graham, MD – H195

Discloses no financial relationships with commercial entities.

Abigail Jacqueline Grande, MPH – H122

Discloses no financial relationships with commercial entities.

Nadia A. Granger, MD – W20

Discloses no financial relationships with commercial entities.

Ignazio Grattagliano, PsyD – I4, I5, I14

Discloses no financial relationships with commercial entities.

Shayna L. Gray, BS – B151

Discloses no financial relationships with commercial entities.

Raquel Green, BS – B130

Discloses no financial relationships with commercial entities.

Tasha Zemrus Greenberg, MD – H17, H85, H149, H159, H172

Discloses no financial relationships with commercial entities.

Ellen M. Greytak, PhD – B105

Discloses no financial relationships with commercial entities.

Catalin Grigoras, PhD – C18, W28

Discloses no financial relationships with commercial entities.

Kyrsten M. Grimes, MA – I28

Discloses no financial relationships with commercial entities.

Lara R.S. Gris, MS – B26

Discloses no financial relationships with commercial entities.

Danielle Grootenboer, JD – W26

Discloses no financial relationships with commercial entities.

Justin Grover, MS – C14

Discloses no financial relationships with commercial entities.

Michelle Groves, MS – E78

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Luca Guarnera, MS – C12

Discloses no financial relationships with commercial entities.

Mark D. Guido, MS – C31

Discloses no financial relationships with commercial entities.

Victoria Guillén, PhD – I12

Discloses no financial relationships with commercial entities.

Wendy M. Gunther, MD – H40

Discloses no financial relationships with commercial entities.

Sharon L. Guo, MD – I40

Discloses no financial relationships with commercial entities.

Avneesh Gupta, MD – H39

Discloses no financial relationships with commercial entities.

Judith A. Gustafson, BS – J1

Discloses no financial relationships with commercial entities.

Richard E. Gutierrez, JD – F28

Discloses no financial relationships with commercial entities.

### H

Lucina Hackman, PhD – A99

Discloses no financial relationships with commercial entities.

Sarah V. Hainsworth, PhD – D2, D4

Discloses no financial relationships with commercial entities.

Amanda R. Hale, MA – S02

Discloses no financial relationships with commercial entities.

Cory Hall, MS – C8

Discloses no financial relationships with commercial entities.

Megan R. Hall, BA – H163

Discloses no financial relationships with commercial entities.

Anthony M. Hallett – W17

Discloses no financial relationships with commercial entities.

Syed W. Hamdani – B35

Discloses no financial relationships with commercial entities.

Boyd Hamilton – W06

Discloses no financial relationships with commercial entities.

Breanne Hamlett – E25

Discloses no financial relationships with commercial entities.

Derek L. Hammond, BA – J6

Discloses no financial relationships with commercial entities.

Greg Hampikian, PhD – B186

Discloses no financial relationships with commercial entities.

Brett E. Harding, MBA – E66

Discloses no financial relationships with commercial entities.

Robert Hargrove, BS – K69

Discloses no financial relationships with commercial entities.

Vikram Harichandran, MS – C3

Discloses no financial relationships with commercial entities.

Sathya Prakash Harihar – B41

Discloses no financial relationships with commercial entities.

Nina A. Harnarine, BSc – J17

Discloses no financial relationships with commercial entities.

LeAnn Michelle Harrel, BS – B119

Discloses no financial relationships with commercial entities.

Anita Roman Hasert, BS – E60

Discloses no financial relationships with commercial entities.

Neal H. Haskell, PhD – W08

Discloses no financial relationships with commercial entities.

Susan Hatters-Friedman, MD – S01, W03

Discloses no financial relationships with commercial entities.

Jonathan Hayes, MD – H195, W19

Discloses no financial relationships with commercial entities.

Michael Alan Hays, MD – H72

Discloses no financial relationships with commercial entities.

Durdica Hazard, PhD – F46

Discloses no financial relationships with commercial entities.

Xuyang He – K44

Discloses no financial relationships with commercial entities.

Joseph T. Hefner, PhD – A101

Discloses no financial relationships with commercial entities.

Bruce J. Heidebrecht – W10

Discloses no financial relationships with commercial entities.

Kelly Heim, PhD – A107

Discloses no financial relationships with commercial entities.

Jakob Heimer, MD – H111

Discloses no financial relationships with commercial entities.

Michael Heninger, MD – H141

Discloses no financial relationships with commercial entities.

Daniella M. Hernandez – B141

Discloses no financial relationships with commercial entities.

Nicholas P. Herrmann, PhD – A122

Discloses no financial relationships with commercial entities.

Susan Seebode Hetzel, BA – D24

Discloses no financial relationships with commercial entities.

Charles M. Heurich, MFS – BS8

Discloses no financial relationships with commercial entities.

Sunil P.A. Hewage – A89

Discloses no financial relationships with commercial entities.

Terry-Dawn Hewitt, LLM – BS1, F27

Discloses no financial relationships with commercial entities.

R. Austin Hicklin, PhD – B95

Discloses no financial relationships with commercial entities.

Jack Hietpas, PhD – B89

Discloses no financial relationships with commercial entities.

Salina Hisham, MSc – A1

Discloses no financial relationships with commercial entities.

Valeria Hofer – E77, H107, H108, H155

Discloses no financial relationships with commercial entities.

Heike Hofmann, PhD – B100

Discloses no financial relationships with commercial entities.

Pamela M. Hofsass, MS – S02

Discloses no financial relationships with commercial entities.

Kiana L. Holbrook, BS – B58

Discloses no financial relationships with commercial entities.

Tiffany A. Hollenbeck, DO – K73

Discloses no financial relationships with commercial entities.

Carley J. Holmes – H49

Discloses no financial relationships with commercial entities.

Anastasia Holobinko, PhD – W11

Discloses no financial relationships with commercial entities.

Emily L. Horrocks, MSc – B57, B139, E4

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

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Mary F. Horvath, MFS – W06

Discloses no financial relationships with commercial entities.

Rachel M. Houston, PhD – B156

Discloses no financial relationships with commercial entities.

Jessica E. Hovingh – B72, B158

Discloses no financial relationships with commercial entities.

Marilyn A. Huestis, PhD – K75, W21

Discloses no financial relationships with commercial entities.

Lurena A. Huffman, BS – W01

Discloses no financial relationships with commercial entities.

Cris E. Hughes, PhD – A106

Discloses no financial relationships with commercial entities.

Nicolas R. Hughes, JD – F37

Discloses no financial relationships with commercial entities.

Cortney N. Hulse, MA – W11

Discloses no financial relationships with commercial entities.

Cheryl D. Hunter – S02

Discloses no financial relationships with commercial entities.

Alyssa Hurd, BA - Y12

Discloses no financial relationships with commercial entities.

Florence Hutner, JD – W26

Discloses no financial relationships with commercial entities.

Shivonne Hutson, MS – W19

Discloses no financial relationships with commercial entities.

Jessica Hvozdozovich, BS – K50

Discloses no financial relationships with commercial entities.

Jessica Hyde, MS – W06

Discloses no financial relationships with commercial entities.

James B. Hyzer, PhD – D19

Discloses no financial relationships with commercial entities.

### I

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Samiah Ibrahim, BSc – J13

Discloses no financial relationships with commercial entities.

Megan E. Ingvaldstad, PhD – A9

Discloses no financial relationships with commercial entities.

Keith Inman, MCrim – W10

Discloses no financial relationships with commercial entities.

Mariyam I. Isa, MA – A139

Discloses no financial relationships with commercial entities.

Carolyn V. Isaac, PhD – H145

Discloses no financial relationships with commercial entities.

Daniel S. Isenschmid, PhD – K70

Discloses no financial relationships with commercial entities.

Hariharan Iyer, PhD – W10

Discloses no financial relationships with commercial entities.

### J

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Ami L. Jackson, DO – H186

Discloses no financial relationships with commercial entities.

John P. Jackson, PhD – E73

Discloses no financial relationships with commercial entities.

Rebecca S. Jackson, MBA – E73

Discloses no financial relationships with commercial entities.

Barry Jansen – W25

Discloses no financial relationships with commercial entities.

Hannah C. Jarvis, MRCS – H69

Discloses no financial relationships with commercial entities.

Gulnaz T. Javan, PhD – B1, H95

Discloses no financial relationships with commercial entities.

Amanda J. Jenkins, PhD – K63

Discloses no financial relationships with commercial entities.

Antaya L. Jennings, BA – A145

Discloses no financial relationships with commercial entities.

Yangeung Jeong, PhD – A6

Discloses no financial relationships with commercial entities.

Karly L. Johannsen – H25

Discloses no financial relationships with commercial entities.

Bryan Johnson, MSFS – W19

Discloses no financial relationships with commercial entities.

Rudolph C. Johnson, PhD – W05

Discloses no financial relationships with commercial entities.

Kellie Jones, BA – A19

Discloses no financial relationships with commercial entities.

Sandra E. Jones, BS – H15

Discloses no financial relationships with commercial entities.

Michelle Jonika – H91

Discloses no financial relationships with commercial entities.

Rachel Joseph, MSc – A7

Discloses no financial relationships with commercial entities.

Morgane Jotterand, MD – W07

Discloses no financial relationships with commercial entities.

Raphaëlle Jouin – I7

Discloses no financial relationships with commercial entities.

Chelsey A. Juarez, PhD – A121

Discloses no financial relationships with commercial entities.

Timothy Juedes, MS – E53

Discloses no financial relationships with commercial entities.

Rebecca Jufer Phipps, PhD – K43

Discloses no financial relationships with commercial entities.

Kun Won Jung, MD, PhD – E46

Discloses no financial relationships with commercial entities.

### K

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Sherri L. Kacinko, PhD – W27

Discloses no financial relationships with commercial entities.

Joseph B. Kadane, PhD – B93

Discloses no financial relationships with commercial entities.

Michal Kaliszan, PhD – H131

Discloses no financial relationships with commercial entities.

Brooke W. Kammrath, PhD – B169, B200

Discloses no financial relationships with commercial entities.

Fehmida Kanodarwala, PhD – B94

Discloses no financial relationships with commercial entities.

Umit Karabiyik, PhD – C32

Discloses no financial relationships with commercial entities.

Andrea Kardohely – E54, E9

Discloses no financial relationships with commercial entities.

Erin L. Karschner, PhD – K62

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Sierra Kaszubinski, BS – H28

Discloses no financial relationships with commercial entities.

Daniel E. Katz, MFS – W23

Discloses no financial relationships with commercial entities.

Rohan P. Kedar, MD – I37

Discloses no financial relationships with commercial entities.

Sharon Kelley, PhD – E88

Discloses no financial relationships with commercial entities.

Philip M. Kemp, PhD – W21

Discloses no financial relationships with commercial entities.

Tyler Kennedy, BS – B154

Discloses no financial relationships with commercial entities.

Linda Kenney Baden, JD – W08

Discloses no financial relationships with commercial entities.

Michael W. Kenyhercz, PhD – A102, W14

Discloses no financial relationships with commercial entities.

Shelby R. Khandasamy, BS – E107

Discloses no financial relationships with commercial entities.

Kazuhiko Kibayashi, MD – H57

Discloses no financial relationships with commercial entities.

Jennifer P. Kidd – B42

Discloses no financial relationships with commercial entities.

Brianna D. Kiesel, BA – B8

Discloses no financial relationships with commercial entities.

Danylle Kightlinger – K11

Discloses no financial relationships with commercial entities.

Eunmi Kim, PhD – K25

Discloses no financial relationships with commercial entities.

Joonyeong Kim, PhD – B143

Discloses no financial relationships with commercial entities.

Victor J. Kim, BBA – K55

Discloses no financial relationships with commercial entities.

Ashley N. Kimble, BS – K19

Discloses no financial relationships with commercial entities.

Sarah C. Kindschuh, PhD – A3

Discloses no financial relationships with commercial entities.

Robert Kirkby, MS – W23

Discloses no financial relationships with commercial entities.

Tetsushi Kitayama, PhD – B129

Discloses no financial relationships with commercial entities.

Alexandra R. Klales, PhD – A43

Discloses no financial relationships with commercial entities.

Megan K. Kleeschulte, MA – H47

Discloses no financial relationships with commercial entities.

Allison C. Klein, BA – H179

Discloses no financial relationships with commercial entities.

Kimberly S. Kobjek, MS – E114

Discloses no financial relationships with commercial entities.

Anthony Koertner, MS – E90, W13

Discloses no financial relationships with commercial entities.

Youngsu Kong – D31

Discloses no financial relationships with commercial entities.

Thomas G. Kopczynski, BS – E57

Discloses no financial relationships with commercial entities.

Marina Korolija, PhD – B188

Discloses no financial relationships with commercial entities.

Elena Kranioti, PhD – A93

Discloses no financial relationships with commercial entities.

Kacy Krehbiel, MD – H44

Discloses no financial relationships with commercial entities.

Rachel M. Kreher, BS – A41, B46

Discloses no financial relationships with commercial entities.

Nico Kresl – C6

Discloses no financial relationships with commercial entities.

Kewal Krishan, PhD – E56

Discloses no financial relationships with commercial entities.

Supriya Krishna, MSc – K31

Discloses no financial relationships with commercial entities.

Robert Kronstrand, PhD – W27

Discloses no financial relationships with commercial entities.

Alex J. Krotulski, MS – K48, S02, W05

Discloses no financial relationships with commercial entities.

John L. Krstenansky, PhD – K32

Discloses no financial relationships with commercial entities.

Raymond Kuk, MS – W18

Discloses no financial relationships with commercial entities.

Kevin P. Kulbacki, MSFS – J7

Discloses no financial relationships with commercial entities.

Shauna Kumar – H42

Discloses no financial relationships with commercial entities.

Sam Kwiatkowski, PhD – B153

Discloses no financial relationships with commercial entities.

Kelsey Kyllonen, MA – B63

Discloses no financial relationships with commercial entities.

Xenia Paula Kyriakou – A136

Discloses no financial relationships with commercial entities.

### L

Ericka N. L'Abbe, PhD – A92, W11

Discloses no financial relationships with commercial entities.

Laura M. Labay, PhD – W27

Discloses no financial relationships with commercial entities.

Mathieu Lacambre, MD – I23

Discloses no financial relationships with commercial entities.

Kimberly E. LaGatta, BS – Y14

Discloses no financial relationships with commercial entities.

Alexis M. LaGoy – A20, A45, A46

Discloses no financial relationships with commercial entities.

Jeffrey Lai, MD – W5

Discloses no financial relationships with commercial entities.

Vienna C. Lam, MA – A78

Discloses no financial relationships with commercial entities.

Taylor Lambrigger – A77

Discloses no financial relationships with commercial entities.

Shannon Lamy – B17

Discloses no financial relationships with commercial entities.

Rachel Lane – E96

Discloses no financial relationships with commercial entities.

Michela Lanfranchi – H104, H107

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Natalie R. Langley, PhD – A73

Discloses no financial relationships with commercial entities.

S.B. Addison Larson, MS – D26

Discloses no financial relationships with commercial entities.

Anna Lasinska – J8

Discloses no financial relationships with commercial entities.

Bartholomeus G.H. Latten, MSc – H113

Discloses no financial relationships with commercial entities.

Troy Lawrence, BBA – C32

Discloses no financial relationships with commercial entities.

Erwan Le Garff, MD – H12

Discloses no financial relationships with commercial entities.

R. Ryan Leahy, MD – I9

Discloses no financial relationships with commercial entities.

Andrea Ledic, BS – J9, J23

Discloses no financial relationships with commercial entities.

Igor K. Lednev, PhD – E82

Discloses no financial relationships with commercial entities.

Henry C. Lee, PhD – W08

Discloses no financial relationships with commercial entities.

You-Rim Lee, BS – E48, E83

Discloses no financial relationships with commercial entities.

F.L. Jim Lee, Jr., MS – J22

Discloses no financial relationships with commercial entities.

Carrie B. LeGarde, MA – A14

Discloses no financial relationships with commercial entities.

Carolyne E. Lemieux, MD – H86

Discloses no financial relationships with commercial entities.

Nikolas P. Lemos, PhD – S02, W27

Discloses no financial relationships with commercial entities.

Yara V. Lemos, MS – A87

Discloses no financial relationships with commercial entities.

John J. Lentini, BA – B90, F26

Discloses no financial relationships with commercial entities.

Janelle Leo, BS – B123

Discloses no financial relationships with commercial entities.

Brandon C. Letts, PhD – W19

Discloses no financial relationships with commercial entities.

Mark M. LeVaughn, MD – H18, H43, H82, H148, W11

Discloses no financial relationships with commercial entities.

Jane A. Lewis, MFS – J24

Discloses no financial relationships with commercial entities.

Russell Lewis, PhD – W21

Discloses no financial relationships with commercial entities.

Joseph L. Lewis III, MFS – E39

Discloses no financial relationships with commercial entities.

Richard Li, PhD – B121

Discloses no financial relationships with commercial entities.

Sun Yi Li, BSc – B131

Discloses no financial relationships with commercial entities.

Linchuan Liao – K67

Discloses no financial relationships with commercial entities.

Aldo Liberto, MD – F33

Discloses no financial relationships with commercial entities.

Julia C. Liebl, BA – K57

Discloses no financial relationships with commercial entities.

Eugene Y. Lien, MS – W10

Discloses no financial relationships with commercial entities.

Sarah Lighthart, BS - Y20

Discloses no financial relationships with commercial entities.

Evelyn S. Ligon – E27

Discloses no financial relationships with commercial entities.

Jennifer F. Limoges, MS – W12

Discloses no financial relationships with commercial entities.

Laura L. Liptai, PhD – W17

Discloses no financial relationships with commercial entities.

Mark A. Listewnik, MA – W15

Discloses no financial relationships with commercial entities.

Ginesse A. Listi, PhD – A98

Discloses no financial relationships with commercial entities.

Steven J. Little, BS – E105

Discloses no financial relationships with commercial entities.

Jianmei Liu – B60

Discloses no financial relationships with commercial entities.

Jeffrey R. Locke, JD – K81

Discloses no financial relationships with commercial entities.

Tracy-Lynn E. Lockwood, BS – E97

Discloses no financial relationships with commercial entities.

Barry K. Logan, PhD – BS4, F20, H136, K46, K81, L1, W05, W08

Discloses no financial relationships with commercial entities.

Holly Long – A42

Discloses no financial relationships with commercial entities.

Cameron M. Longo – B29

Discloses no financial relationships with commercial entities.

Kim M. Look, DDS – E73

Discloses no financial relationships with commercial entities.

Dayanira Lopez, BS – A64

Discloses no financial relationships with commercial entities.

Jennifer C. Love, PhD – A72, S02

Discloses no financial relationships with commercial entities.

Jiaqi M. Lu, MS – E61

Discloses no financial relationships with commercial entities.

Victoria S. Lucas, PhD – G19

Discloses no financial relationships with commercial entities.

Ashley M. Luebbers, MS – H71

Discloses no financial relationships with commercial entities.

Francesco Lupariello, MD – E12, E14

Discloses no financial relationships with commercial entities.

Vincenzo Lusa, JD – F2, I3, LW2

Discloses no financial relationships with commercial entities.

Zhou Lyu, PhD – H102

Discloses no financial relationships with commercial entities.

### M

Andrea Macarulla, MSc – C24

Discloses no financial relationships with commercial entities.

William A. MacCrehan, PhD – B88

Polydimethylsiloxane (PDMS) (Discussion of Unlabeled/  
Investigational Use of Product/Device).

Mitsuyo Machida – E47

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Francesca Maglietta, MD – E4, E8, E9, E18, E62, E70, H58

Discloses no financial relationships with commercial entities.

Zahid Mahmood, MPhil – B20

Discloses no financial relationships with commercial entities.

Christopher A. Maier, PhD – A117

Discloses no financial relationships with commercial entities.

Sergey Mamedov, PhD – B167

Discloses no financial relationships with commercial entities.

João Manata, MSc – E67

Discloses no financial relationships with commercial entities.

Rachel Maragliano – B189

Discloses no financial relationships with commercial entities.

Michael Marciano, MS – B157

Discloses no financial relationships with commercial entities.

Mollie Mares, MSFS – B137

Discloses no financial relationships with commercial entities.

Luisa Marinho, MSc – A49

Discloses no financial relationships with commercial entities.

Michael A. Markey, MD – H170

Discloses no financial relationships with commercial entities.

Mike Markushewski, BS – D38

Discloses no financial relationships with commercial entities.

Maricla Marrone, MD – G31

Discloses no financial relationships with commercial entities.

Daniel A. Martell, PhD – S01

Discloses no financial relationships with commercial entities.

Rosa M. Martinez, MD – H107, H108, H155

Discloses no financial relationships with commercial entities.

Kelsey Mason, BS – H179

Discloses no financial relationships with commercial entities.

Rebecca A. Mastrovito, MS – K78

Discloses no financial relationships with commercial entities.

Nivia A.M. Matias, MD – H160

Discloses no financial relationships with commercial entities.

Wojciech Matkowski – C38, C39

Discloses no financial relationships with commercial entities.

Tomotaka Matsubara – D13

Discloses no financial relationships with commercial entities.

Ashleigh K. Matzoll – B47

Discloses no financial relationships with commercial entities.

Brendan P. Max, JD – F28

Discloses no financial relationships with commercial entities.

Henry P. Maynard III, MSFS – E116

Discloses no financial relationships with commercial entities.

Maria Carla Mazzotti, MD – E31

Discloses no financial relationships with commercial entities.

Thomas C. McAndrew, MA – BS8

Discloses no financial relationships with commercial entities.

Jared E. McAtee - Y16

Discloses no financial relationships with commercial entities.

Anna G. McDonald, MD – H13

Discloses no financial relationships with commercial entities.

Gary McDonald, Jr., JD – F25

Discloses no financial relationships with commercial entities.

Nathan R. McFadden - Y6

Discloses no financial relationships with commercial entities.

Jessica McFarland – E100

Discloses no financial relationships with commercial entities.

James McGivney, DMD – G23

Discloses no financial relationships with commercial entities.

Courtney K. McGowan, BS – K21

Discloses no financial relationships with commercial entities.

Jonathan G. McGrath, PhD – E113

Discloses no financial relationships with commercial entities.

Wayne J. McKenna, LLB – BS1

Discloses no financial relationships with commercial entities.

Heather E. McKiernan, MSFS – B80, L1

Discloses no financial relationships with commercial entities.

Patrick McLaughlin, BS – B114

Discloses no financial relationships with commercial entities.

Chelsey McPhillen – C6

Discloses no financial relationships with commercial entities.

Chris Meffert, MS – C14

Discloses no financial relationships with commercial entities.

Samantha A. Mehnert – E24

Discloses no financial relationships with commercial entities.

Federica Mele, MD - I17

Discloses no financial relationships with commercial entities.

Niccolò D. Melloni, MD – E3

Discloses no financial relationships with commercial entities.

Sydney Menchhoff – B112

Discloses no financial relationships with commercial entities.

Julian L. Mendel, PhD – B7

Discloses no financial relationships with commercial entities.

Vera Mendes-Kramer, MA – H39

Discloses no financial relationships with commercial entities.

Edidiong Mendie – E41

Discloses no financial relationships with commercial entities.

Christina Rose Mendralla, BS – K65

Discloses no financial relationships with commercial entities.

M.J. Menendez, JD – W05, F19, F20, K81

Discloses no financial relationships with commercial entities.

Korina Menking-Hoggatt, MSc – E108

Discloses no financial relationships with commercial entities.

Stephen B. Mercer, JD – F5

Discloses no financial relationships with commercial entities.

Elena Mercuri – H36

Discloses no financial relationships with commercial entities.

Rizky Merdieto Boedi, MSc – G16

Discloses no financial relationships with commercial entities.

Mara L. Merlino, PhD – J2, J6, J11, J31

Discloses no financial relationships with commercial entities.

Rodolfo Mesa, BS – B140

Discloses no financial relationships with commercial entities.

Vadim Mesli, MD – H115

Discloses no financial relationships with commercial entities.

Diana L. Messer, MS – H106

Discloses no financial relationships with commercial entities.

Mitchell Messner, BA – H128

Discloses no financial relationships with commercial entities.

Roger D. Metcalf, DDS, JD – LW1

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Abigail L. Meyer, BS – E95

Discloses no financial relationships with commercial entities.

Katarzyna Michaud, MD – H129, W07

Discloses no financial relationships with commercial entities.

Suzanna Michener, MSc – A30

Discloses no financial relationships with commercial entities.

Suzanne Miles, BS – E74, E76

Discloses no financial relationships with commercial entities.

Jessica Miller, BS – B124

Discloses no financial relationships with commercial entities.

Lauren Miller, PhD – I27

Discloses no financial relationships with commercial entities.

James Millette, PhD – D7

Discloses no financial relationships with commercial entities.

Colleen F. Milligan, PhD – A79

Discloses no financial relationships with commercial entities.

George R. Milner, PhD – W24

Discloses no financial relationships with commercial entities.

Heather V. Milnthorp, MSFS – B116

Discloses no financial relationships with commercial entities.

Randolph L. Mitchell, DMD – G30

Discloses no financial relationships with commercial entities.

Cara A. Mitrano - Y9

Discloses no financial relationships with commercial entities.

Rhonda M. Mittenzwei, MD – H56

Discloses no financial relationships with commercial entities.

Summer M. Mizell – A42

Discloses no financial relationships with commercial entities.

Carmen Silvia M. Miziara, MD, PhD – G10

Discloses no financial relationships with commercial entities.

Audris Mockus, PhD – C37

Discloses no financial relationships with commercial entities.

Mariah E. Moe, BS – A125

Discloses no financial relationships with commercial entities.

Amanda L.A. Mohr, MSFS – K47

Discloses no financial relationships with commercial entities.

Gruschenka Mojica Sanchez, MD – H168

Discloses no financial relationships with commercial entities.

Benjamin Mokdad – E36

Discloses no financial relationships with commercial entities.

Cristina Mondello, MD – H33

Discloses no financial relationships with commercial entities.

Angelo Montana, MD – H83

Discloses no financial relationships with commercial entities.

Dennise Montero, BS – B104

Discloses no financial relationships with commercial entities.

Romain Montoriol, MD – B30

Discloses no financial relationships with commercial entities.

Marykathryn Tynon Moody, MSFS – K34

Discloses no financial relationships with commercial entities.

Clarra G. Moore, BS – J3

Discloses no financial relationships with commercial entities.

Konstantinos Moraitis, PhD – A9, A17

Discloses no financial relationships with commercial entities.

Jessica Morel, DO – I22

Discloses no financial relationships with commercial entities.

Sarah V. Morello – E106

Fuji IR Camera; Sirchie ALS Units (Discussion of Unlabeled/ Investigational Use of Product/Device).

Matteo Moretti, MD – K66

Discloses no financial relationships with commercial entities.

Daniel J. Morgan, MS – E64

Discloses no financial relationships with commercial entities.

Michael A. Mosco, MS – E55

Discloses no financial relationships with commercial entities.

Sharon K. Moses, PhD – E122

Discloses no financial relationships with commercial entities.

Nikki Mourtzinis, DO – H112

Discloses no financial relationships with commercial entities.

Autumn T. Muise – H29

Discloses no financial relationships with commercial entities.

Marzena H. Mulawka, MFS – E55

Discloses no financial relationships with commercial entities.

Carmen T. Mulet, BS – K39

Discloses no financial relationships with commercial entities.

Adam Munera – A133

Discloses no financial relationships with commercial entities.

Bala Muralikrishnan, PhD – C40

Discloses no financial relationships with commercial entities.

Daniel C. Murrie, PhD – E120

Discloses no financial relationships with commercial entities.

Rabi A. Musah, PhD – E124

Discloses no financial relationships with commercial entities.

### N

Nidal Nabhan Abou, MD – I21

Discloses no financial relationships with commercial entities.

Harman Nahal, BSc – A140

Discloses no financial relationships with commercial entities.

Marcela Najarro, MFS – B202, W23

Discloses no financial relationships with commercial entities.

Aden G. Naka, MSc – W19

Discloses no financial relationships with commercial entities.

John B. Nase, DDS – G11, G25

Discloses no financial relationships with commercial entities.

Yolanda Nerkowski, BA – G40

Discloses no financial relationships with commercial entities.

Janelle D.S. Newman, MD – B173

Discloses no financial relationships with commercial entities.

Jennifer Newman, PhD – C17

Discloses no financial relationships with commercial entities.

Kia K. Newman, MD – E94

Discloses no financial relationships with commercial entities.

Matthew N. Newmeyer, PhD – K29

Discloses no financial relationships with commercial entities.

Georgina R. Nicholls, MS – B180

Discloses no financial relationships with commercial entities.

John Nixon, MBA - D5, D6

Discloses no financial relationships with commercial entities.

Karen J. Nobles, BA – J25

Discloses no financial relationships with commercial entities.





## Presenting Author Financial Disclosure – 2019

Wanda Nocerino – F41

Discloses no financial relationships with commercial entities.

Francy Scarlett Nogales, BS – B130

Discloses no financial relationships with commercial entities.

Diana C. Nointin, MD – H3

Discloses no financial relationships with commercial entities.

Jessica T. Novak, BA – A19

Discloses no financial relationships with commercial entities.

Carolina Núñez-Vázquez, PhD – H34

Discloses no financial relationships with commercial entities.

W. Milton Nuzum III, JD – F44

Discloses no financial relationships with commercial entities.

Emilio Nuzzolese, PhD – G1, G9, G36, G39, G41

Discloses no financial relationships with commercial entities.

### O

Kerry J. O'Connell, JD – F23

Discloses no financial relationships with commercial entities.

Craig O. O'Connor, PhD – W26

Discloses no financial relationships with commercial entities.

Heather M. O'Connor, DO – H64

Discloses no financial relationships with commercial entities.

Nicole R. Odom, BS – C20

Discloses no financial relationships with commercial entities.

Edwin O. Olaya Molina, BA – E33, E69

Discloses no financial relationships with commercial entities.

Fabio Oldoni, PhD – B108

Discloses no financial relationships with commercial entities.

Laura C. Oliver, MS – B84

Discloses no financial relationships with commercial entities.

Bianca E. Olivieri – K76

Discloses no financial relationships with commercial entities.

Stephanie A. Olson – E50

Discloses no financial relationships with commercial entities.

Ayomide Oludoyi, BSc – H174

Discloses no financial relationships with commercial entities.

Danica Ommen, PhD – B159, J26

Discloses no financial relationships with commercial entities.

Kelly C. O'Neill – B91

Discloses no financial relationships with commercial entities.

Kathryn L. Orton, BS – B176

Discloses no financial relationships with commercial entities.

Andrea Ost, MS – A38

Discloses no financial relationships with commercial entities.

Alessio Ostuni, MD – I35

Discloses no financial relationships with commercial entities.

Colby Ott, BS – K38

Discloses no financial relationships with commercial entities.

Stephen D. Ousley, PhD – A26, W24

Discloses no financial relationships with commercial entities.

Oriana Ovide – B19

Discloses no financial relationships with commercial entities.

### P

Tyrish Y. Page, MA – E1

Discloses no financial relationships with commercial entities.

Chiara Palazzo, MD – H2

Discloses no financial relationships with commercial entities.

Andrea Palmiotto, PhD – A13

Discloses no financial relationships with commercial entities.

Kaitlyn B. Palmquist, BS – K17

Discloses no financial relationships with commercial entities.

Ariana Pape, BA – H54

Discloses no financial relationships with commercial entities.

Donna M. Papsun, MS – W05

Discloses no financial relationships with commercial entities.

Antonino Paratore, MS – C12, F41

Discloses no financial relationships with commercial entities.

Manoj Bhausaheb Parchake, MD – H162

Discloses no financial relationships with commercial entities.

Dae-Kyoon Park, MD, PhD – E52

Discloses no financial relationships with commercial entities.

Kevin J. Parmelee, PhD – W25

Discloses no financial relationships with commercial entities.

Christian T. Pascual – K23

Discloses no financial relationships with commercial entities.

Nicholas V. Passalacqua, PhD – A69

Discloses no financial relationships with commercial entities.

Federico Patanè, MD – F49

Discloses no financial relationships with commercial entities.

Simmi Patel, BS – I29

Discloses no financial relationships with commercial entities.

Michael A. Peat, PhD – W15

Discloses no financial relationships with commercial entities.

Jennifer L. Pechal, PhD – H98

Discloses no financial relationships with commercial entities.

Kimberly Peek, BSN – E13

Discloses no financial relationships with commercial entities.

Anne E. Perez, PhD – W08

Discloses no financial relationships with commercial entities.

Dorians Mercedes Perez, MS – A103

Discloses no financial relationships with commercial entities.

Mark W. Perlin, PhD, MD – B186, F45

Discloses no financial relationships with commercial entities.

Katelynn A. Perrault, PhD – E51, H93

Discloses no financial relationships with commercial entities.

Diane C. Peterson, MD – W20

Discloses no financial relationships with commercial entities.

Caterina Petetta, MD – E3, E5

Discloses no financial relationships with commercial entities.

Pierre-Antoine Peyron, MD – H67

Discloses no financial relationships with commercial entities.

Amy Pham, BA – A51

Discloses no financial relationships with commercial entities.

Angelina I. Phillips, MD – E1, H51

Discloses no financial relationships with commercial entities.

Richard Phillips, BA – A36

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Sandra M. Pieczonka - Y18

Discloses no financial relationships with commercial entities.

Jennifer Piel, MD – S01

Discloses no financial relationships with commercial entities.

David Pienkowski, PhD – D37

Discloses no financial relationships with commercial entities.

Marin A. Pilloud, PhD – A146, W22

Discloses no financial relationships with commercial entities.

Christine M. Pink, PhD – A22

Discloses no financial relationships with commercial entities.

Jason M. Piotrowski, BA – W05

Discloses no financial relationships with commercial entities.

Deborah C. Pinto, PhD – A70

Discloses no financial relationships with commercial entities.

Haskell M. Pitluck, JD – W08

Discloses no financial relationships with commercial entities.

Sharon L. Plotkin, MS – W02

Discloses no financial relationships with commercial entities.

Mateus D.C. Polastro, MSc – C9, C11, C15

Discloses no financial relationships with commercial entities.

Carrie Polston, BA – J15

Discloses no financial relationships with commercial entities.

Cristoforo Pomara, MD, PhD – F49

Discloses no financial relationships with commercial entities.

Katharine Chapman Pope, MA – H144

Discloses no financial relationships with commercial entities.

David J. Porta, PhD – D40

Discloses no financial relationships with commercial entities.

Vanessa Potkin, JD – BS7

Discloses no financial relationships with commercial entities.

Mark C. Pozzi, MS – D18, D22, D27

Discloses no financial relationships with commercial entities.

Joseph A. Prahlow, MD – H191, H195

Discloses no financial relationships with commercial entities.

Sebastien S. Prat, MD – I6, I22

Discloses no financial relationships with commercial entities.

Rich Press, MEdSc – W10

Discloses no financial relationships with commercial entities.

DeMia P. Pressley, MS – B64, K81

Discloses no financial relationships with commercial entities.

Jana M. Price, PhD – W21

Discloses no financial relationships with commercial entities.

Mechthild K. Prinz, PhD – B113

Discloses no financial relationships with commercial entities.

Emily Priszaznik, MS - Y8

Discloses no financial relationships with commercial entities.

Noemi Procopio, PhD – A60

Discloses no financial relationships with commercial entities.

Lori A. Proe, DO – H137

Discloses no financial relationships with commercial entities.

Keith E. Propp, PhD – E73

Discloses no financial relationships with commercial entities.

Abigail J. Props, MS – H100

Discloses no financial relationships with commercial entities.

Meghan Prusinowski – B59, B133

Discloses no financial relationships with commercial entities.

Jane Pucher, JD – BS7

Discloses no financial relationships with commercial entities.

Giovanna Punzi – I38

Discloses no financial relationships with commercial entities.

### Q

Lawrence Quarino, PhD – E118

Discloses no financial relationships with commercial entities.

Ana Luiza Queiroz, BA – E59

Discloses no financial relationships with commercial entities.

Matthew Quinn – B168

Olympus BX35F Polarized Light Microscope, Thermo-Fisher Scientific DXR2 Raman Microscope (Discussion of Unlabeled/ Investigational Use of Product/Device).

Jan Ynav T. Quiz – H70

Discloses no financial relationships with commercial entities.

### R

Prem Rachakonda – C40

Discloses no financial relationships with commercial entities.

Roberto Raffaele, BE – E91, I39, H152, K26

Discloses no financial relationships with commercial entities.

Christopher W. Rainwater, MS – A124

Discloses no financial relationships with commercial entities.

Imron G. Ramos, BS – E21

Discloses no financial relationships with commercial entities.

Donald J. Ramsell, JD – F21

Discloses no financial relationships with commercial entities.

Katherine Ramsland, PhD – E68, W02

Discloses no financial relationships with commercial entities.

Anjali A. Ranadive, JD – W08

Discloses no financial relationships with commercial entities.

Isabelle R. Raquin, LLM – F5

Discloses no financial relationships with commercial entities.

Jean-Sébastien Raul – D16

Discloses no financial relationships with commercial entities.

Manasa Ravoori, BDS – G15

Discloses no financial relationships with commercial entities.

Sophie Raymond, MD – I19

Discloses no financial relationships with commercial entities.

Abdul Razaq, MPhil – E86

Discloses no financial relationships with commercial entities.

Walter M. Reaves, JD – F16

Discloses no financial relationships with commercial entities.

Karen Reczek, MLS – BS3

Discloses no financial relationships with commercial entities.

Stacey L. Reed, DO – H14, H16

Discloses no financial relationships with commercial entities.

Gowri V. Reesu, MSc – G26

Discloses no financial relationships with commercial entities.

Jacqueline S. Reid, DDS – G34

Discloses no financial relationships with commercial entities.

Christoph Reisinger, MD – E72

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Marcello Rendine, DBA – B146, E80

Discloses no financial relationships with commercial entities.

Kevin Riach, JD – F40

Discloses no financial relationships with commercial entities.

Kiegan Rice, MS – E85

Discloses no financial relationships with commercial entities.

Jason D. Ricke, JD – F38

Discloses no financial relationships with commercial entities.

Michael F. Rieders, PhD – BS4

Discloses no financial relationships with commercial entities.

Irene Riezzo, MD, PhD – H11

Discloses no financial relationships with commercial entities.

Sarah Riman, PhD – B110

Discloses no financial relationships with commercial entities.

Michelle Rippy – E115

Discloses no financial relationships with commercial entities.

Heidy P. Rivera, BA – K30

Discloses no financial relationships with commercial entities.

David B. Rivers, PhD – H88

Discloses no financial relationships with commercial entities.

Leann G. Rizer, BS – F7

Discloses no financial relationships with commercial entities.

Vittorio Rizzi – F41

Discloses no financial relationships with commercial entities.

Zackery Roberson, BS – B181

Discloses no financial relationships with commercial entities.

Graham J. Roberts, MDS – G21

Discloses no financial relationships with commercial entities.

Elizabeth Robinson, MS – B25

Discloses no financial relationships with commercial entities.

Haley Rock, BS – B46

Discloses no financial relationships with commercial entities.

Sandra E. Rodriguez-Cruz, PhD – B170

Discloses no financial relationships with commercial entities.

Marcus Rogers, PhD – S02

Discloses no financial relationships with commercial entities.

Melissa Rogers - Y10

Discloses no financial relationships with commercial entities.

Meghan N. Roig, MSFS – B115

Discloses no financial relationships with commercial entities.

Cristin Marie Rolf, MD – LW5

Discloses no financial relationships with commercial entities.

Maxwell O. Rollins, MD – H9

Discloses no financial relationships with commercial entities.

Madeline G. Roman, BS – B155

Discloses no financial relationships with commercial entities.

Megan Romano, DVM – E84

Discloses no financial relationships with commercial entities.

Erica L. Romsos, MFS – B190

Discloses no financial relationships with commercial entities.

Valentina Ronco, MD – F12, H24

Discloses no financial relationships with commercial entities.

Catherine Rood, BS – H177

Discloses no financial relationships with commercial entities.

Jeri D. Roper-Miller, PhD – K81

Discloses no financial relationships with commercial entities.

Karen B. Rosenbaum, MD – S01

Discloses no financial relationships with commercial entities.

Terri Rosenblatt, JD – F43

Discloses no financial relationships with commercial entities.

Ann H. Ross, PhD – A39

Discloses no financial relationships with commercial entities.

Claude Roux, PhD – E119

Discloses no financial relationships with commercial entities.

Samantha K. Rowbotham, MArSc – A86

Discloses no financial relationships with commercial entities.

Katie M. Rubin, MS – A15

Discloses no financial relationships with commercial entities.

Jacqueline Ruchti, BS – B67

Discloses no financial relationships with commercial entities.

Ralph A. Ruffolo, BSc – D32

Discloses no financial relationships with commercial entities.

Lexus R. Rutter, BS – K61

Discloses no financial relationships with commercial entities.

Colbey Ryman – E57

Discloses no financial relationships with commercial entities.

### S

Sara Sabatasso, MD – W07

Discloses no financial relationships with commercial entities.

Matteo A. Sacco, MD – E91, E111, H150, H152, K26

Discloses no financial relationships with commercial entities.

Geetanjali Sachdeva, MSc – E109

Discloses no financial relationships with commercial entities.

Mark E. Safarik, MS – E68

Discloses no financial relationships with commercial entities.

William Sago, MD – H178

Discloses no financial relationships with commercial entities.

Tomoya Sakuragawa – D14

Discloses no financial relationships with commercial entities.

Zeenat Salahuddin, PhD – B148

Discloses no financial relationships with commercial entities.

Monica Salerno, MD, PhD – E7, E70, F49

Discloses no financial relationships with commercial entities.

Amy Salyards - Y17

Discloses no financial relationships with commercial entities.

David San Pietro, PhD – B200

Discloses no financial relationships with commercial entities.

M. Isabel Sanchez-Melo, MS – J32

Discloses no financial relationships with commercial entities.

Ilaria Santoiemma – E6, H153, I16

Discloses no financial relationships with commercial entities.

Alessandro Santurro, MD – H158

Discloses no financial relationships with commercial entities.

Rosalina Intan Saputri, DDS, MSc – G14

Discloses no financial relationships with commercial entities.

Hrvoje Sarajlija, PhD – E102

Discloses no financial relationships with commercial entities.

Julianne J. Sarancho, MS – A76

Discloses no financial relationships with commercial entities.



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Laura Sare – E117

Discloses no financial relationships with commercial entities.

Shelby M. Sarginson – K33

Discloses no financial relationships with commercial entities.

Adrienne E. Sauder, MD – H66

Discloses no financial relationships with commercial entities.

Kelly Sauerwein, PhD – A84

Discloses no financial relationships with commercial entities.

Christopher P. Saunders, PhD – B159

Discloses no financial relationships with commercial entities.

Yih Ling Saw, MSFS – E42

Discloses no financial relationships with commercial entities.

Samantha J. Sawyer, BS – E117

Discloses no financial relationships with commercial entities.

Christopher J. Scallon, MS – W01

Discloses no financial relationships with commercial entities.

Juliette Scantlebury, MD – W20

Discloses no financial relationships with commercial entities.

Madison R. Schackmuth, BS – K24

Discloses no financial relationships with commercial entities.

Kristin Schelling, MS – W26

Discloses no financial relationships with commercial entities.

Lisa C. Schiermeier-Wood, MS – W10

Discloses no financial relationships with commercial entities.

John D. Schmid, JD – W26

Discloses no financial relationships with commercial entities.

Gregory Schmit, MD – H52

Discloses no financial relationships with commercial entities.

Lynn A. Schneeweis, MS – F10

Discloses no financial relationships with commercial entities.

Leigh-Ann Schuerman, DMD – G20

Discloses no financial relationships with commercial entities.

Thorsten Schwark, MD – H161

Discloses no financial relationships with commercial entities.

Andrew J. Schweighardt, PhD – B39

Discloses no financial relationships with commercial entities.

Wolf Schweitzer – H87, H104, H107, H108, H109, H154, H155

Discloses no financial relationships with commercial entities.

Matteo Scopetti, MD – H37

Discloses no financial relationships with commercial entities.

G. Richard Scott, PhD – W22

Discloses no financial relationships with commercial entities.

Veronica Scotti, LLM – F4, F35

Discloses no financial relationships with commercial entities.

Jan Seaman Kelly, BA – J21

Discloses no financial relationships with commercial entities.

Ismail M. Sebetan, MD, PhD – B66, B70, E5, E21, E9, E99, F11

Discloses no financial relationships with commercial entities.

Jagmahender Singh Sehrawat, PhD – A90, G17

Discloses no financial relationships with commercial entities.

Andrew C. Seidel, MA – A4

Discloses no financial relationships with commercial entities.

Kathryn C. Seigfried-Spellar, PhD – C10

Discloses no financial relationships with commercial entities.

Elizabeth M. Sepulveda, BA – H38

Discloses no financial relationships with commercial entities.

Serenella Serinelli, MD – H55, H73, H116, H123, H125, H139

Discloses no financial relationships with commercial entities.

Francesco Sessa, MS – E8, E9, E80, E98, G36

Discloses no financial relationships with commercial entities.

Puneet Setia, MD – H185

Discloses no financial relationships with commercial entities.

Cassandra Hernandez Setser, MSFS – B118

Discloses no financial relationships with commercial entities.

Francesco Servida – C22

Discloses no financial relationships with commercial entities.

Arian Dokht Shahmirza, BSc – C35

Discloses no financial relationships with commercial entities.

Nathan S. Shaller, MD – H126

Discloses no financial relationships with commercial entities.

Aaron M. Shapiro, PhD – K59

Discloses no financial relationships with commercial entities.

Amy T. Sheil, MD – ES1

Discloses no financial relationships with commercial entities.

Donald E. Shelton, JD, PhD – F4

Discloses no financial relationships with commercial entities.

David T. Sheppard, PhD – W18

Discloses no financial relationships with commercial entities.

Kevin E. Sheridan, PhD – A45, A46, A47

Discloses no financial relationships with commercial entities.

Alissa M. Shida, BA – E17

Discloses no financial relationships with commercial entities.

Mary K. Shields, DMD – G38

Discloses no financial relationships with commercial entities.

Vivian Shnaidman, MD – I42

Discloses no financial relationships with commercial entities.

Elisa N. Shoff, BS – K49, S02

Discloses no financial relationships with commercial entities.

Francesco Sicilia, MD – H150, H151, K26

Discloses no financial relationships with commercial entities.

Sarah J. Sides, BS – H174

Discloses no financial relationships with commercial entities.

Michael E. Sigman, PhD – B102

Discloses no financial relationships with commercial entities.

Mylene A. Signori, BSc – J12

Discloses no financial relationships with commercial entities.

Asit Kumar Sikary, MD – H134, H182, K68

Discloses no financial relationships with commercial entities.

Terrie Simmons-Ehrhardt, MA – A59

Discloses no financial relationships with commercial entities.

Alison G. Simon, PhD – B61, B162

Discloses no financial relationships with commercial entities.

Rochelle A. Simon, MD – H175

Discloses no financial relationships with commercial entities.

Baneshwar Singh, PhD – B154

Discloses no financial relationships with commercial entities.

Monika Singh, MSc – G29

Mitutoyo digital caliper (Discussion of Unlabeled/

Investigational Use of Product/Device).

Chiara Siodambro – H36

Discloses no financial relationships with commercial entities.



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Edward Sisco, PhD – W23

Discloses no financial relationships with commercial entities.

Stephanie R. Sivak, MS – E79

Discloses no financial relationships with commercial entities.

Jennifer A. Sizemore – A136

Discloses no financial relationships with commercial entities.

Jessica Slater – E44

Discloses no financial relationships with commercial entities.

Beth M. Slaybaugh, MPS – K4

Discloses no financial relationships with commercial entities.

Alycia M. Smentkiewicz – E106

Fuji IR camera; Sirchie ALS Units (Discussion of Unlabeled/ Investigational Use of Product/Device).

Alexander J. Smith, BA – A56

Discloses no financial relationships with commercial entities.

Daniel G. Smith, MA – W12

Discloses no financial relationships with commercial entities.

Erich D. Smith, MS – B99

Discloses no financial relationships with commercial entities.

Gabriella Smith, BS – Y11

Discloses no financial relationships with commercial entities.

Jeff M. Smith, MS – C25, W06

Discloses no financial relationships with commercial entities.

Juliette Smith – B52

Discloses no financial relationships with commercial entities.

La'Quida Smith, MA – J6

Discloses no financial relationships with commercial entities.

Patricia C. Smith, MSL – BS2

Discloses no financial relationships with commercial entities.

Rachel E. Smith – A109

Discloses no financial relationships with commercial entities.

Nurtami Soedarsono, PhD – G2

Discloses no financial relationships with commercial entities.

Biagio Solarino, PhD – H68

Discloses no financial relationships with commercial entities.

Angela Soler, PhD – A105, W19

Discloses no financial relationships with commercial entities.

Tessa Somogyi, MA – A20, A45, A46

Discloses no financial relationships with commercial entities.

Renee Sorrentino, MD – I22, W03

Discloses no financial relationships with commercial entities.

Miriam E. Soto Martinez, PhD – A141

Discloses no financial relationships with commercial entities.

Richard R. Souvion, DDS – G6

Discloses no financial relationships with commercial entities.

Patricia M. Speck, DNSc – E13

Discloses no financial relationships with commercial entities.

Caroline Spencer, BS – K54

Discloses no financial relationships with commercial entities.

Micayla C. Spiros, MS – A100

Discloses no financial relationships with commercial entities.

Kate Spradley, PhD – A144

Discloses no financial relationships with commercial entities.

Supawon Srettabunjong, MD – H138

Discloses no financial relationships with commercial entities.

Trevor I. Stamper, PhD – E23, H97

Discloses no financial relationships with commercial entities.

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Sharon K. Stanford, BA – W12

Discloses no financial relationships with commercial entities.

Dawnie W. Steadman, PhD – A32

Discloses no financial relationships with commercial entities.

Becky Steffen, MS – B191

Discloses no financial relationships with commercial entities.

Paul Stein, PhD – B66, B70, E5, E21, E39, E99, F11

Discloses no financial relationships with commercial entities.

Hannah Stephen, BS – A55

Discloses no financial relationships with commercial entities.

Molly B. Still, BS – H31

Discloses no financial relationships with commercial entities.

Reinoud D. Stoel, PhD – J34

Discloses no financial relationships with commercial entities.

Andrew Stolbach, MD – W05

Discloses no financial relationships with commercial entities.

Anne Stone, PhD – BS5

Discloses no financial relationships with commercial entities.

Jonah W.P. Stone, BS – B46

Discloses no financial relationships with commercial entities.

David A. Stoney, PhD – B97

Discloses no financial relationships with commercial entities.

Phoebe R. Stubblefield, PhD – A147

Discloses no financial relationships with commercial entities.

Emma L. Stuhmer, BS – B15

Discloses no financial relationships with commercial entities.

Aishwaryaa Subramanian – B45

Discloses no financial relationships with commercial entities.

Jolee T. Suddock, DO – H173

Discloses no financial relationships with commercial entities.

Khlea Sumani, DMD – G35

Discloses no financial relationships with commercial entities.

Robert L. Sumwalt III, MS – W21

Discloses no financial relationships with commercial entities.

Eddie Surer, MD – H107

Discloses no financial relationships with commercial entities.

Joel D. Sutton, MSFS – W10

Discloses no financial relationships with commercial entities.

Cassandra A. Swart, BS – B34, K12

Discloses no financial relationships with commercial entities.

Mary C. Swearingner, MA – A114

Discloses no financial relationships with commercial entities.

Jenna K. Sweet – B11

Discloses no financial relationships with commercial entities.

Henry J. Swofford, MSFS – W13, W16

Discloses no financial relationships with commercial entities.

Steven A. Symes, PhD – W08, W11

Discloses no financial relationships with commercial entities.

### T

Sabrina C. Ta'ala, MA – A132

Discloses no financial relationships with commercial entities.

Daniela Tafur – E33

Discloses no financial relationships with commercial entities.

Mohammad A. Tahir, PhD – E86, J20

Discloses no financial relationships with commercial entities.



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Mio Takayama, PhD – H165

Discloses no financial relationships with commercial entities.

Chikako Takei – K1

Discloses no financial relationships with commercial entities.

Sean D. Tallman, PhD – A143

Discloses no financial relationships with commercial entities.

Jessica Tang, BS – B126

Discloses no financial relationships with commercial entities.

Zeying Tang, LLM – H102

Discloses no financial relationships with commercial entities.

Caroline Tanguay, MD – G12

Discloses no financial relationships with commercial entities.

Francesca Tarantino, MD – G31

Discloses no financial relationships with commercial entities.

Esiri Tasker, BA – B163

Discloses no financial relationships with commercial entities.

John Tavalacci, BS – C8

Discloses no financial relationships with commercial entities.

Jonee Taylor, MD – H81

Discloses no financial relationships with commercial entities.

Melissa K. Taylor, MA – J34, W10

Discloses no financial relationships with commercial entities.

Caryn E. Tegtmeier, PhD – A16

Discloses no financial relationships with commercial entities.

Keith-Dane H. Temporal, MS – B132

Discloses no financial relationships with commercial entities.

MariaTeresa A. Tersigni-Tarrant, PhD – A73, W20

Discloses no financial relationships with commercial entities.

Marissa A. Teske – K40

Discloses no financial relationships with commercial entities.

Chelsie N. Testerman, MS – B82

Discloses no financial relationships with commercial entities.

Warren D. Tewes, DDS – LW4

Discloses no financial relationships with commercial entities.

Inci Y. Tezbasan, BSc – E110, I36

Discloses no financial relationships with commercial entities.

Michael Thali, MD – H87, H104, H107, H108, H155

Discloses no financial relationships with commercial entities.

Patrick W. Thevissen, PhD – G1

Discloses no financial relationships with commercial entities.

Jeff Thomas, MBA – E1

Discloses no financial relationships with commercial entities.

Richard M. Thomas, PhD – W19

Discloses no financial relationships with commercial entities.

Christopher R. Thompson, MD – S01

Discloses no financial relationships with commercial entities.

Wanna Thongnoppakhun – H138

Discloses no financial relationships with commercial entities.

Wantawanop Thongphap, BSc – H138

Discloses no financial relationships with commercial entities.

Drake Ryan Thrasher, BS – H78, H176

Discloses no financial relationships with commercial entities.

Nicholas A. Thurn – E103

Discloses no financial relationships with commercial entities.

Teresa M Tiedge – B107

Discloses no financial relationships with commercial entities.

Miranda R. Tilton, BA – C1

Discloses no financial relationships with commercial entities.

Nicholas B. Tiscione, MS – K9

Discloses no financial relationships with commercial entities.

Justine M. Titko, MSFS – K64

Discloses no financial relationships with commercial entities.

Samantha Tolliver, PhD – K51

Discloses no financial relationships with commercial entities.

Jeffery K. Tomberlin, PhD – BS7, E117

Discloses no financial relationships with commercial entities.

Joseph Toomey, PhD – I27

Discloses no financial relationships with commercial entities.

Jan J. Topoleski, MFS – A95

Discloses no financial relationships with commercial entities.

Mario Torreggianti, DDS – G39

Discloses no financial relationships with commercial entities.

Michelle N. Torres, BS – B147

Discloses no financial relationships with commercial entities.

Lauri Traub, JD – W25

Discloses no financial relationships with commercial entities.

Silvia Trotta – H68, K15

Discloses no financial relationships with commercial entities.

Nilesh K. Tumram, MD – H6, H166

Discloses no financial relationships with commercial entities.

Sara Turco – E15, E65

Discloses no financial relationships with commercial entities.

Rachelle A. Turiello, MS – B4

Discloses no financial relationships with commercial entities.

Jane W. Turner, PhD, MD – W20

Discloses no financial relationships with commercial entities.

### U

Noelle J. Umback, PhD – W19

Discloses no financial relationships with commercial entities.

Evi Untoro, MD – A91

Discloses no financial relationships with commercial entities.

Petra Urbanová, PhD – E38

Discloses no financial relationships with commercial entities.

Paul Uribe, MD – H140, H181

Discloses no financial relationships with commercial entities.

Tugba Ünsal, PhD – B144

Discloses no financial relationships with commercial entities.

Samantha Upton, BA – Y22

Discloses no financial relationships with commercial entities.

### V

Peter R. Valentin, MSFS – E35

Discloses no financial relationships with commercial entities.

Julie L. Valentine, PhD – E74, E76

Discloses no financial relationships with commercial entities.

Raymond Valerio, JD – F43

Discloses no financial relationships with commercial entities.

Michael K. Valle, BS – B128

Discloses no financial relationships with commercial entities.



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Peter M. Vallone, PhD – W10

Discloses no financial relationships with commercial entities.

Evan Van Leer Greenberg, JD – S01

Discloses no financial relationships with commercial entities.

Valerie Van Leer Greenberg, JD – S01

Discloses no financial relationships with commercial entities.

Rick R. Van Rijn, PhD – H105, H117

Discloses no financial relationships with commercial entities.

Jan Peter van Zandwijk, PhD – H8

Discloses no financial relationships with commercial entities.

Joshua Vandeburgh – H45

Discloses no financial relationships with commercial entities.

Courtney H. Vander Pyl – B19

Discloses no financial relationships with commercial entities.

Susan VanderPlas – E58

Longitudinal Shoe Outsole Database (Discussion of

Unlabeled/Investigational Use of Product/Device).

Oluseyi A. Vanderpuye, PhD – K42

Discloses no financial relationships with commercial entities.

Jessica Vanhaebost – H52

Discloses no financial relationships with commercial entities.

Jessica Vanhaebost – H110

Angiofil (Discussion of Unlabeled/Investigational Use of

Product/Device).

Elena Varotto – E9

Discloses no financial relationships with commercial entities.

Thomas W. Vastrick, BS – J5, S02

Discloses no financial relationships with commercial entities.

Mark Vecellio, MFS – E106

Fuji IR camera; Sirchie ALS (Discussion of Unlabeled/

Investigational Use of Product/Device).

Elvira Ventura Spagnolo, MD – H33, H157

Discloses no financial relationships with commercial entities.

Marloes E.M. Vester, MD – H7, H194

Discloses no financial relationships with commercial entities.

Giovanna M. Vidoli, PhD – F36

Discloses no financial relationships with commercial entities.

Katelin Vietri, BA - Y13

Discloses no financial relationships with commercial entities.

Svante Vikingsson, PhD – K60

Discloses no financial relationships with commercial entities.

Greg Vincent, MD – K80

Discloses no financial relationships with commercial entities.

Silvia D. Visona, MD – H62

Discloses no financial relationships with commercial entities.

Eleanor B. Vo, MD – I22

Discloses no financial relationships with commercial entities.

Caitlin C.M. Vogelsberg, MS – A118, F9

Discloses no financial relationships with commercial entities.

Laura Volpini, PhD – E34

Discloses no financial relationships with commercial entities.

Joelle V. Vuille, PhD – F29, F46

Discloses no financial relationships with commercial entities.

## W

Crystal L. Wagoner, MFS – E89

Discloses no financial relationships with commercial entities.

Mark A. Waldo, MFS – E99

Discloses no financial relationships with commercial entities.

Stewart Walker, PhD – B203

Discloses no financial relationships with commercial entities.

Mackenzie Walls – A119

Discloses no financial relationships with commercial entities.

Brittany S. Walter, PhD – A37

Discloses no financial relationships with commercial entities.

Leah Walton, BA – W21

Discloses no financial relationships with commercial entities.

Rachel Hamilton Walton, EdD – W15

Discloses no financial relationships with commercial entities.

Alick Wang, BSc – I10

Discloses no financial relationships with commercial entities.

Amber R. Wang, MD – H4

Discloses no financial relationships with commercial entities.

Ling Wang, MS – B21

Discloses no financial relationships with commercial entities.

Margaret Warner, PhD – K81

Discloses no financial relationships with commercial entities.

Dione Washington – E40

Discloses no financial relationships with commercial entities.

Brian Joseph Waters, MS – K8

Discloses no financial relationships with commercial entities.

Jenna M.S. Watson, MA – A57

Discloses no financial relationships with commercial entities.

Steven B. Watson, BA – C7, W06

Discloses no financial relationships with commercial entities.

Rebecca A. Waxse, JD – S02

Discloses no financial relationships with commercial entities.

Jennifer L. Webb, BS – A19

Discloses no financial relationships with commercial entities.

Alexis R. Weber, BS – B22

Discloses no financial relationships with commercial entities.

Nicholas Webster, MD – W21

Discloses no financial relationships with commercial entities.

Vicki Wedel, PhD – A18

Discloses no financial relationships with commercial entities.

Victor W. Weedn, MD, JD – BS4, F30, W17

Discloses no financial relationships with commercial entities.

Svenja Weise, PhD – W24

Discloses no financial relationships with commercial entities.

Katherine E. Weisensee, PhD – A23

Discloses no financial relationships with commercial entities.

Kurt D. Weiss, MS – D36, D39

Discloses no financial relationships with commercial entities.

Steven Weitz, MS – B78

Discloses no financial relationships with commercial entities.

Karin E. Wells, BA – H23

Discloses no financial relationships with commercial entities.

Hannah K. Weppner - Y4

Discloses no financial relationships with commercial entities.



## Presenting Author Financial Disclosure – 2019

Joshua White, BS – H120

Discloses no financial relationships with commercial entities.

Teresa A. White, MA – W02

Discloses no financial relationships with commercial entities.

Ray Wickenheiser, MBA – W10

Discloses no financial relationships with commercial entities.

Emily F. Wieggers, MA – A21

Discloses no financial relationships with commercial entities.

Carl Wigren, MD – W03

Discloses no financial relationships with commercial entities.

Rachel E. Wiley, MFS – B86

Discloses no financial relationships with commercial entities.

Emily J. Will, MA – J34

Discloses no financial relationships with commercial entities.

David A. Williams, DDS – B36

Discloses no financial relationships with commercial entities.

Joyce P. Williams, DNP – B36

Discloses no financial relationships with commercial entities.

Loren M. Williams, BS – J28

Adobe® Photoshop®, BioTuring (Discussion of Unlabeled/  
Investigational Use of Product/Device).

Sheila Willis, PhD – W10

Discloses no financial relationships with commercial entities.

Taylor J. Willis – I26

Discloses no financial relationships with commercial entities.

Gregoreese Willocks – B14

Discloses no financial relationships with commercial entities.

Bailey M. Wills, BS – B111

Discloses no financial relationships with commercial entities.

Teresa V. Wilson, PhD – A97

Discloses no financial relationships with commercial entities.

Rebecca J. Wilson-Taylor, PhD – A16

Discloses no financial relationships with commercial entities.

Allysha P. Winburn, PhD – A145

Discloses no financial relationships with commercial entities.

Janis Winchester, MS – J19

VP-8 Image Analyzer (Discussion of Unlabeled/Investigational  
Use of Product/Device).

Agnes D. Winokur, MS – BS4

Discloses no financial relationships with commercial entities.

Andrew J. Winter, MS – B68

Discloses no financial relationships with commercial entities.

Eric D. Wish, PhD – W05

Discloses no financial relationships with commercial entities.

David Witzke, BA – G22

Discloses no financial relationships with commercial entities.

Michael Wixted, MA – W12

Discloses no financial relationships with commercial entities.

Denise Wohlfahrt, BS – B85, B130, H32

Discloses no financial relationships with commercial entities.

Emily Wolak, DO – H164

Discloses no financial relationships with commercial entities.

Cydnee A. Wolfe - Y2

Discloses no financial relationships with commercial entities.

Rebecca Wood, MFS – E123

Discloses no financial relationships with commercial entities.

Robert E. Wood, DDS, PhD – G5, G24, G40

Discloses no financial relationships with commercial entities.

Taylor A. Wood – B23

Discloses no financial relationships with commercial entities.

Grace S. Woods, BS – H133

Discloses no financial relationships with commercial entities.

Charlotte J. Word, PhD – F43, W10

Discloses no financial relationships with commercial entities.

Franklin D. Wright, DMD – G4

Discloses no financial relationships with commercial entities.

Patrick M. Wright – E106

Fuji IR camera; Sirchie ALS Lights (Discussion of Unlabeled/  
Investigational Use of Product/Device).

Jingjie Wu, PhD – K20

Discloses no financial relationships with commercial entities.

Sulin Wu, PhD – H183

Discloses no financial relationships with commercial entities.

Charis Wynn, MSc – K52

Discloses no financial relationships with commercial entities.

### Y

Vamsi Yadavalli, PhD – B125

Discloses no financial relationships with commercial entities.

Aylin Yalçın Sarıbey, PhD – B13

Discloses no financial relationships with commercial entities.

Chu-An Yang, MS – K14, K77

Discloses no financial relationships with commercial entities.

Duygu Yavuz – F31

Discloses no financial relationships with commercial entities.

Dana M. Yenke, BS – B50

Discloses no financial relationships with commercial entities.

Hatice Yilmaz, BSc – F3

Discloses no financial relationships with commercial entities.

Leena Yoon – B12

Discloses no financial relationships with commercial entities.

Kenichi Yoshizawa, MPharm – E104

Discloses no financial relationships with commercial entities.

John L. Young, MD – I8

Discloses no financial relationships with commercial entities.

Elizabeth Young Laanemets, MSc - W5

Discloses no financial relationships with commercial entities.

### Z

Jorge J. Zacca – K79

Discloses no financial relationships with commercial entities.

Carlos J. Zambrano, PhD – H156

Discloses no financial relationships with commercial entities.

Victoria Zeger – E22

Discloses no financial relationships with commercial entities.

Ellyn A. Zeidman, BS – B44

Discloses no financial relationships with commercial entities.

Mengliang Zhang, PhD – B150

Discloses no financial relationships with commercial entities.

Mingchang Zhang – H184

Discloses no financial relationships with commercial entities.





## Presenting Author Financial Disclosure – 2019

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Ning Zhang – D1

Discloses no financial relationships with commercial entities.

Shuangteng Zhang, PhD – C30, C34

Discloses no financial relationships with commercial entities.

Xiaoyu A. Zheng, MS – B99

Discloses no financial relationships with commercial entities.

Andrew D. Ziegler, BS – B83

Discloses no financial relationships with commercial entities.

Debra Prince Zinni, PhD – A68

Discloses no financial relationships with commercial entities.

James Zjalic, MSc – C33

Discloses no financial relationships with commercial entities.



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